Spring 4-24-2020

The Effects of Temperature on Evacuation Rates and Absorption Efficiency of Flathead Catfish

Zach Horstman
University of Nebraska - Lincoln, zachary.horstman@huskers.unl.edu

THE EFFECTS OF TEMPERATURE ON EVACUATION RATES AND ABSORPTION EFFICIENCY OF FLATHEAD CATFISH

By

Zachary D. Horstman

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Natural Resource Sciences

Under the Supervision of Professor Jamilynn B. Poletto

Lincoln, Nebraska

May 2020

THE EFFECTS OF TEMPERATURE ON EVACUATION RATES AND ABSORPTION EFFICIENCY OF FLATHEAD CATFISH

Zachary D. Horstman M.S.

University of Nebraska, 2020

Advisor: Jamilynn B. Poletto

Knowledge of fish gastric evacuation rates are a necessary component for both field and laboratory studies when trying to understand feeding rates, modeling energy budgets, and understanding trophic dynamics of aquatic ecosystems. Many freshwater fish encounter a broad range of environmental temperatures across life history stages, but the mechanistic link between temperature and physiological processes often remains poorly understood. We designed three recirculating aquatic systems capable of rearing Flathead Catfish *Plyodictis olivaris* in order to quantify gastric evacuation rates and gross energy absorption at three temperatures (17º C, 22º C and 25º C). We examined the relationship between temperature and its influences on consumption, gastric evacuation rates, and meal passage through the intestines of juvenile Flathead Catfish fed a single ration of food. We then used bomb calorimetry to examine the absorption efficiencies of juvenile Flathead Catfish by quantifying the proportion of calories remaining in dissected stomach and intestinal contents through time and by treatment. Temperature significantly affected consumption, gastric evacuation rates, and the amount of calories remaining in the intestinal contents of juvenile Flathead Catfish. Insight into the gastric evacuation rates of juvenile Flathead Catfish provides managers with a better understanding of consumption demands of these important top predators. Furthermore, our results begin to shed light on the bioenergetics of juvenile Flathead Catfish.

Acknowledgments

I would first like to thank my wife, Carly, for all the love and support she has provided me as I have pursued my passion for fisheries. She has been a positive light throughout the process and my number one supporter. I am so grateful for her patience, understanding, sense of humor, and positive attitude throughout this journey. I would truly not be where I am today without her by my side.

I would like to thank my advisor, Dr. Jamilynn Poletto, who has supported me and provided me with this opportunity to develop and grow as a researcher. I want to thank my committee members, Dr. Mark Pegg and Dr. Marty Hamel, for their help throughout my academic career; you have both provided me with opportunities to succeed within the field. Thank you Dr. Kevin Pope, Dr. Chris Chizinski, Dennis Ferraro and countless other faculty for helping shape my understanding of applied ecology and statistical methods. I am grateful for all the invaluable lessons I have learned from each of you.

Thank you to the staff at the Nebraska Game and Parks Commission who have been supportive of me in my time working there. Thank you, Rick Holland and Thad Huenemann, for the opportunity to begin my career while finishing my graduate program. I would also like to thank Joe Spooner and all the staff in the Missouri River and Southeast District office for their encouragement.

I am grateful for the close-knit community that the University of Nebraska – Lincoln and the School of Natural Resources has provided me. Thank you to my

lab mate and friend, Alex Engel, for your jovial attitude, giving nature, inquisitive questions, and constant support. I have learned a great deal working with you, and from you, over the past few years. Thank you to Israt Jahan, Annie Madsen, and Lyndsie Wszola for your support and help within the Poletto Lab. Thank you to my classmates Esther Perisho, Jake Werner, Quintin Dean, Henry H. Hansen, Lindsay Ohlman, Dave Keiter, Derek Kane, Brittany Buchanan, and many other students that I have shared experiences with in and out of the classroom. Thank you to the past cohorts of students within the Pegg lab that have provided me with great examples to learn from within the fisheries profession. Thank you to the lab technicians Octavious Bartek, Jessie Hall, Cat Berrick, and Ryan Thomas for all your help with late night fish dissections and constant fish husbandry assistance. This journey would not have been possible without all of you.

Finally, I would like to thank my family and friends who have helped me throughout my educational career. My passion for the outdoors began long before I imagined pursuing a career in fisheries, so I appreciate the support you gave to help get me to where I am now. I cannot thank you all enough for the love and shared experiences that have shaped me into the person I am today.

Table of Contents

LIST OF TABLES IV

LIST OF FIGURES VII

LIST OF APPENDICES X

CHAPTER 1: GENERAL INTRODUCTION AND STUDY OBJECTIVES 1

LITERATURE CITED 8

CHAPTER 2: THE EFFECTS OF TEMPERATURE ON GASTRIC EVACUATION RATES AND GUT PASSAGE TIME OF FLATHEAD CATFISH *PLYODICTIS OLIVARIS* 14

ABSTRACT 14

INTRODUCTION 15

METHODS 19

RESULTS 30

DISCUSSION 37

LITERATURE CITED 45

CHAPTER 3: THE EFFECTS OF TEMPERATURE ON ABSORPTION EFFICIENCIES OF FLATHEAD CATFISH *PLYODICIS OLIVARIS* 79

ABSTRACT 79

INTRODUCTION 80

METHODS 85

RESULTS 92

DISCUSSION 97

LITERATURE CITED 103

GENERAL CONCLUSIONS 121

APPENDICES 124

LIST OF TABLES

Table 2-1. Summary information of the mean total length (mm) and weight (g) of Flathead Catfish, mean Goldfish weight (g), and mean ration size (meal size as % body weight of Flathead Catfish), count of male, female, and unknown Flathead Catfish among all treatments combined (n = 210 Flathead Catfish). 51

Table 2-2. Results of an ANOVA (α = 0.05) of sex, age, and temperature on gonadosomatic index values of Flathead Catfish..... 52

Table 2-3. Summary information of water quality parameters. Mean and standard deviation (SD) of ammonia, nitrite, nitrate and pH levels for each system throughout the duration of the experiment. Differences among means were tested with ANOVA (α = 0.05)..... 53

Table 2-4. Generalized linear model (family binomial) selection, using Akaike information criterion (AICc), for consumption of Goldfish by juvenile Flathead Catfish. We examined temperature (º C), prey weight (wet weight of Goldfish), and experimental tanks as predictors of consumption. 54

Table 2-5. Examination of two supporting models on consumption of Goldfish by juvenile Flathead Catfish. Differences among means were tested with ANOVA (α = 0.05). 55

Table 2-6. Results of a post-hoc analysis using least square means for multiple comparison (Tukey-adjusted comparison) to examine differences in consumption between treatments (17º C, 22º C, 25º C). Mean consumption events between treatments are different based on α = 0.05. 56

Table 2-7. Generalized linear model (family binomial) selection, using Akaike information criterion (AICc), for gastric evacuation rates of juvenile Flathead Catfish. We examined digestion time (hrs), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), experimental tank, sex, and age as predictors of the proportion of the meal remaining in the stomach (wet weight of stomach content divided by initial wet weight of Goldfish) of juvenile Flathead Catfish..... 57

Table 2-8. Results of an ANOVA (α= 0.05) of digestion time, temperature, and ration size on the proportion of the meal remaining (wet weight of stomach contents divided by initial wet weight of Goldfish fed to Flathead Catfish) in the stomachs of juvenile Flathead Catfish. 58

Table 2-9. Results of a post-hoc analysis using least square means for multiple comparison (Tukey-adjusted comparison) to examine differences in gastric evacuation rates between treatments (17º C, 22º C, 25º C) of juvenile Flathead Catfish. Mean gastric evacuation rates between treatments were different based on α = 0.05. 59

Table 2-10. Estimated time (hours) to reach a given percentage of stomach evacuation for juvenile Flathead Catfish. Gastric emptying time (time at which the stomach is empty) was based on a 90% evacuation of the meal from the stomach and is shown in bold. 60

Table 3-1. Generalized linear model (family Gaussian) selection, using Akaike information criterion (AICc), for calories/g dry weight remaining within the stomach of juvenile Flathead Catfish. We examined digestion time (hr), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), sex, Flathead Catfish length, and experimental tanks as predictors of the calories/g dry weight remaining in the stomachs of juvenile Flathead Catfish. 108

Table 3-2. Generalized linear model (family Gaussian) selection, using Akaike information criterion (AICc), for calories/g dry weight remaining within the intestines of juvenile Flathead Catfish. We examined digestion time (hrs), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), sex, Flathead Catfish length, and experimental tanks as predictors of the calories/g dry weight remaining in the intestines of juvenile Flathead Catfish. 109

Table 3-3. Results of an ANOVA (α= 0.05) of digestion time (hr) and treatment temperature (17º C, 22º C, and 25º C) on the calories/g dry weight remaining in the intestines of juvenile Flathead Catfish. 110

Table 3-4. Results of a post-hoc analysis using least square means for multiple comparison (Tukey-adjusted comparison) to examine differences in calories/g dry weight remaining in the intestines of juvenile Flathead Catfish among treatments (17º C, 22º C, 25º C). Mean calories/g dry weight were different between treatments based on α = 0.05. 111

Table 3-5. Generalized linear model (family binomial) selection, using Akaike information criterion (AICc), for the proportion of gross calorie absorption throughout the entire alimentary canal of juvenile Flathead Catfish. We examined digestion time (hrs), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), experimental tank, sex, and age as predictors of the proportion of gross calorie absorbed. 112

Table 3-6. Results of an ANOVA (α= 0.05) of digestion time (hr), treatment temperature (17º C, 22º C, and 25º C), and ration size (meal size as a proportion of Flathead Catfish body weight) on the proportion of gross calorie absorption (total calories assessed in stomach and intestines divided by initial predicted calories fed) of juvenile Flathead Catfish. 113

Table 3-7. Results of an ANOVA (α= 0.05) of digestion time (hr), treatment temperature (17º C, 22º C, and 25º C), and ration size (meal size as a proportion of Flathead Catfish body weight) on the proportion of gross calorie absorption (total calories assessed in stomach and intestines divided by initial predicted calories fed) of juvenile Flathead Catfish restricted to 2-16 hours post consumption. 114

LIST OF FIGURES

Figure 2-1. Conceptual diagram of the recirculating aquatic system within the University of Nebraska - Lincoln Fish Conservation, Behavior, and Physiology Laboratory. 64

Figure 2-2. Conceptual diagram of random distribution of Flathead Catfish (n = 210) among three treatment (17º C, 22º C, and 25º C) groups. Tank D of each system was left empty for experimental aquaria setup. 65

Figure 2-3. Conceptual diagram of the experimental aquaria setup in tank D of a recirculating aquatic system. Water flowed freely between the experimental aquaria and the surrounding tank. A Flathead Catfish and a Goldfish of known wet weight were placed within an experimental aquarium. Cameras above each experimental aquaria were used to monitor consumption. 66

Figure 2-4. A photo of the digestive tract of a Flathead Catfish. Food passes from the esophagus into the stomach before passing into intestines and expelling from the anus. The solid line indicates where the stomach empties into the intestines. 67

Figure 2-5. Box and whisker plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of temperature among tanks of each system. Temperature was not different among tanks within each system; system 1 ($F_{3,532} = 0.026$, $P = 0.994$), system 2 ($F_{3,532} = 0.059$, $P = 0.981$), and system 3 ($F_{3,420} = 0.481$, $P = 0.696$). Differences were tested with ANOVA ($\alpha = 0.05$). 68

Figure 2-6. Box and whisker plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of dissolved oxygen (mg/L) among tanks of each system. Dissolved oxygen was different among tanks within each system; system 1 ($F_{3,532} = 11.61$, $P < 0.001$), system 2 ($F_{3,532} = 6.631$, $P < 0.001$), and system 3 ($F_{3,420} = 2.809$, $P = 0.039$). Differences were tested with ANOVA ($\alpha = 0.05$). 69

Figure 2-7. Box plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of Flathead Catfish weights (g) for each treatment. Flathead Catfish weight was not different ($F_{2,123} = 0.374$, $P = 0.689$) among treatments. Differences were tested with ANOVA ($\alpha = 0.05$). 70

Figure 2-8. Box plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of initial Goldfish weights (g) that were consumed by Flathead Catfish for each treatment. Goldfish weights were not different ($F_{2,123} =$

0.283, P = 0.754) among treatments. Differences were tested with ANOVA ($\alpha = 0.05$)..... 71

Figure 2-9. Box plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of ration sizes that were consumed by Flathead Catfish for each treatment. Goldfish weights were not different ($F_{2,123} = 0.945$, P = 0.392) among treatments. Differences were tested with ANOVA ($\alpha = 0.05$)..... 72

Figure 2-10. Bar graph (with mean and 95% CI) representing the proportion of consumption (number of Flathead Catfish that consumed a Goldfish divided by the total number of trials) that occurred within each treatment temperature (17º C, 22º C, and 25º C). Consumption of Goldfish increased as temperatures increased. A proportion of 0.429 (± 0.111) of Flathead Catfish ate at 17º C, 0.594 (± 0.120) of Flathead Catfish ate at 22º C, and .809 (± 0.093) of Flathead Catfish ate at 25º C. There was a significant difference between 17º C and 25º C (P < 0.001) and 22º C and 25º C (P = 0.021) treatments, but not 17º C and 22º C (P = 0.127)..... 73

Figure 2-11. Binomial logistic regression for each treatment (17º C, solid line; 22º C, dashed line; and 25º C, dotted line) on the proportion of the meal remaining in stomach of juvenile Flathead Catfish (n =126); based on nine digestion times (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h) and a mean ration size of 0.015. Regression overlays the proportion of the meal remaining in the stomach of dissected individuals (17º C, squares, n = 33; 22º C, circles, n = 38; and 25º C, triangles, n = 55). Gastric evacuation rates were different between 17º C and 25º C (P = 0.002), but not 17º C and 22º C (P = 0.076) and 22º C and 25º C (P = 0.490)..... 74

Figure 2-12. Binomial logistic regression on the proportion of the meal remaining in intestines of juvenile Flathead Catfish (n =126); based on nine digestion times (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h) and a mean ration size of 0.015. Regression overlays the proportion of the meal remaining in the intestines of dissected individuals (17º C, squares, n = 33; 22º C, circles, n = 38; and 25º C, triangles, n = 55). 75

Figure 2-13. Box plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) on the number of meals consumed per hour interval by juvenile Flathead Catfish during lights off and lights on in the laboratory. 76

Figure 2-14. Bar graph showing the cumulative count of juvenile Flathead Catfish that consumed a Goldfish for each hour of the day. Dashed blue lines indicate lights on (0545) and lights off (1745) in the laboratory. Of the 126 Flathead Catfish to consume a Goldfish, 28 individuals ate during lights on and 98 individuals ate during lights off. 77

Figure 2-15. An empirical cumulative distribution function (ECDF) illustrating all 126 trials that resulted in a juvenile Flathead Catfish consuming a Goldfish and the time of day that consumption events occurred most frequently (steeper slope). Dashed blue lines indicate lights on (0545) and lights off (1745) in the laboratory. 78

Figure 3-1. A simple linear regression showing the strong linear relationship between dry weight and wet weight of Goldfish using a subsample of 28 individuals. 115

Figure 3-2. A simple linear regression showing no relationship between dry weight and calories/g dry weight of Goldfish using a subsample of 17 individuals. 116

Figure 3-3. Generalized linear model of the calories/g dry weight remaining in stomach of juvenile Flathead Catfish (y') based on digestion time (t) and ration size (Rs). Individuals bombed stomach contents are shown for each temperature treatment (17º C = squares, 22º C = circles, and 25º C= triangles). Ration size was held constant with the mean value 0.015 to predict the regression. 117

Figure 3-4. Generalized linear model of the back transformed calories/g dry weight remaining in intestines of juvenile Flathead Catfish (y') based on digestion time (t) and treatment temperature (17º C, solid line; 22º C, dashed line; 25º C, dotted line). T_{22} and T_{25} are binomial inputs (0 or 1) used to examine treatment temperature 22º C (T_{22} = 1 and T_{25} = 0) and 25º C (T_{22} = 0 and T_{25} = 1), 17º C is the reference group T_{22} = 0 and T_{25} = 0. Individuals bombed intestine contents are shown for each temperature treatment (17º C, squares; 22º C, circles; and 25º C, triangles). 118

Figure 3-5. Binomial logistic regression for each treatment (17º C, solid line; 22º C, dashed line; and 25º C, dotted line) on the proportion of the gross calories absorbed by juvenile Flathead Catfish (n =126); based on all nine digestion times (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h) and a mean ration size of 0.015. Regression overlays the proportion of the gross calories absorbed by individuals (17º C, squares, n = 30; 22º C, circles, n = 35; and 25º C, triangles, n = 50). The proportion of gross calorie absorption was not different among treatments ($\alpha = 0.05$). 119

Figure 3-6. Binomial logistic regression for each treatment (17º C, solid line; 22º C, dashed line; and 25º C, dotted line) on the proportion of the gross calories absorbed by juvenile Flathead Catfish (n =126); based on restricted digestion times (2h, 4h, 6h, 8h, 12h, 16h) and a mean ration size of 0.015. Regression overlays the proportion of the gross calories absorbed by individuals (17º C, squares, n = 18; 22º C, circles, n = 23; and 25º C, triangles, n = 31). The proportion of gross calorie absorption was not different among treatments ($\alpha = 0.05$) 120

LIST OF APPENDICES

Appendix 1. A note on rearing Flathead Catfish *Plyodictis olivaris* in a recirculating aquatic system..... 124

Appendix 2. Summary information tables for Chapter 2..... 145

Appendix 3. Summary of wet weight to dry weight conversion and caloric content of Goldfish subsample. 153

Appendix 4. Length weight relationship of sampled juvenile Flathead Catfish and examination of relative weight (Wr) among treatments. 154

Appendix 5. Raw data of each dissected Flathead Catfish in Chapter 2 and Chapter 3..... 156

CHAPTER 1: GENERAL INTRODUCTION AND STUDY OBJECTIVES

Temperature elicits the most control over fish compared to any other abiotic factor (Beitinger and Fitzspatric 1979) and is considered the abiotic master factor (Fry 1971; Brett 1971). Fish live within a medium of high heat capacity and conductance; their body temperature is regulated within a few fractions of a degree of the water temperature (Gunn 1942). Therefore, the rates of all their biological functions are dependent on the temperature of the environment (Jobling 1997). Extensive work has been conducted on the effects of temperature on numerous aspects of fish behavior and physiology. Temperature influences fish physiology by setting lethal limits, limiting movement due to increased metabolic demands, and affecting the metabolic capacities of individuals (Brett 1956).

A fish's ability to navigate waters and encounter preferred temperatures is vital to promoting biological processes that benefit condition and survival (Boltaña et al. 2017). Temperature approaching the upper maximum limit cause high physiological demands and stress in fish as well as reduce oxygen levels in the water (Jackson et. al. 2001). Lower temperature limits can adversely affect a species by preventing sufficient growth. Preferred optimal temperatures affect species distribution (Van Zuiden et al. 2016; Payne et al. 2016) and increase consumption and growth rates (O'Gorman et al. 2016).

Optimal temperatures differ by species, life-history stage, and among physiological processes (Pedersen and Jobling 1989; Somero 2002), resulting in trade-offs between performance. For example, Handeland et al. (2008) found that the overall growth rate of Atlantic salmon, *Salmo salar,* smolts were highest at 14° C while food conversion efficiency was optimized at 10° C. Thus, there is a growing need to understand how temperature (affected by seasonal changes, anthropogenic alterations, and climate change) affects poikilotherms and their physiological processes, particularly consumption and growth.

Metabolism is a series of chemical processes within an organism necessary to sustain life. Gillooly et al. (2001) states, "temperature governs metabolism through its effects on rates of biochemical reactions." Food demand, regulated by peptides in the brain, is a direct function of a species metabolic rate (Moyle and Cech 2004). In order to keep up with this growing demand for energy to support cellular function, fish increase oxygen and nutritional uptake (Helfman et al. 2009). Fish use approximately 40% of dietary energy to maintain life processes (Brett and Groves 1979). Approximately 2-7% of energy is lost through urine and 20-40% is lost through feces (Brett and Groves 1979). Only once the needs of metabolism are met will energy be allocated toward production of fish tissue.

Temperature effects consumption and the processing of energy by influencing digestion, metabolism, egestion, excretion, and swimming speed (Hartman and Brandt 1995). Energy, in terms of kilocalories needed for survival

and growth, increases exponentially over the temperature niche for a given species, while maximum consumption typically increases to a peak (or a plateau) before declining over the range of temperatures a species can tolerate (Jobling 1993; Zweifel et al. 1999). A decline in consumption after its peak indicates a temperature threshold after which growth starts to be constrained regardless of food available in the environment (Bourret et al. 2008).

Managers of aquatic systems benefit in understanding effects of temperature on consumption and growth of a species for a multitude of reasons. Managers can maximize production and promote good health based on observed "optimum" temperature ranges for specific species (Viadero 2005) which is beneficial in aquaculture. Furthermore, consumption rates and the factors governing feeding rates, such as temperature, are important in studies examining fish growth, population dynamics, and the behavior of individuals (Kapoor 1975). For example, locomotor performances vary across temperatures resulting in differences between attack speeds of predator and the escape speeds of prey (Grigaltchik et al. 2005; Allan et al. 2015). Temperature effects competition among fish species by controlling food consumption and aggression (Taniguchi et al. 1998; Carmona-Catot et al. 2013).

Calculating the rate at which a ration passes the alimentary canal provides a way to estimate consumption. Specifically, the gastric evacuation rate of an individual is the rate at which the stomach empties after consuming a meal and the gastric emptying time is the amount of time (h) it takes the stomach to

completely empty, post consumption. It is assumed that over longer periods, the gastric evacuation rate is equal to the rate at which food is being consumed (Seyhan and Grove 2003). Several methods are available to study gastric evacuation rates and include methods such as serial slaughter (Başçınar et al. 2016) and gastric lavage (Sweka et al. 2004). Multiple variables have been known to effect gastric evacuation rates (Bromley 1988; He and Wurtsbaugh 1993), including mass of predator (Andersen 1999), type of prey item (Windell and Norris 1969), mass of prey item (Legler et al. 2010), and temperature (Perrson 1979; Jobling 1980; Nakagawa 2018).

Evacuation rates focus on the breakdown of ingested materials, or digestion. A large portion of digestion occurs within the stomach (Smith 1980). Smaller compounds such as proteins, fats, and carbohydrates complete digestion within the intestines and are then absorbed into the bloodstream (Molnar and Gair 2015). Absorption, defined as nutrient uptake through cell walls, primarily occurs within the intestines (Smith 1980).

Much like food webs try to model energy flow through an ecosystem, the study of bioenergetics is used to understand how an organism uses the energy it consumes. Bioenergetics models provide a framework for understanding how energy is allocated within organisms rather than a precise predictor of what will happen with that energy (Helfman et al. 2009). Bioenergetics models are based on a balanced equation. Therefore, energy allocated toward metabolism, waste,

storage, and growth will equal that of energy consumed (Winberg 1956). The general bioenergetics model can be shown as:

$$C = (R + A + SDA) + F + U + G,$$

where consumed food (i.e., daily rations), *C*, equals the summation of metabolic needs (standard metabolism, *R*; active metabolism, *A*; and specific dynamic action, *SDA*); waste due to egestion through feces, *F*; excretion (i.e. urine), *U*; and growth (somatic and/or gonadal), *G* (Deslauriers 2017). Bioenergetics models can be used to provide information to broaden the context of fish bioenergetics, ultimately pushing forward the discipline of physiology (Jørgensen et al. 2016).

Energy balance is a crucial component in an organism's ability to survive and reproduce. Small variations in the environment of any form may significantly alter how energy is processed or allocated to other forms. Temperature has a profound impact on energy allocation within an organism. The mechanistic link between temperature and physiological processes often remains poorly understood for many organisms. We aim to add to the growing literature of bioenergetics by providing an increased understanding of the effects of temperature on evacuation rates and absorption efficiency of juvenile Flathead Catfish, *Pylodictis olivaris*.

Flathead Catfish are a warm water species (Bourret et al. 2008) native to the Mississippi, Missouri, and Rio Grande river basins (Pflieger 1997; Fuller and Whelan 2018). However, Flathead Catfish have dispersed through natural range expansion and unauthorized introduction in many systems east of the

Appalachians and west of the Rocky Mountains (Fuller et al. 1999). Flathead Catfish are opportunistic feeders and are nonselective, preying upon species that are abundant in their surroundings (Pine et al. 2005; Turner 2017). Therefore, many studies have examined the potential negative impact non-native Flathead Catfish have on native fauna (Pine et al. 2007; Dobbins et al. 2012).

Diet varies based on life history stage, with Flathead Catfish eating primarily insect larvae before becoming more aggressive piscivores around 250 mm (Minckley and Deacon 1959; Roell and Orth 1993). Flathead Catfish reach sexual maturity by age three, or around 400 mm in total length (Hrabik et al. 2015). Flathead Catfish can reach sizes over 1500 mm and weigh more than 45 kg (Hrabik et al. 2015).

Relatively little is known of how temperature effects consumption and absorption efficiency of juvenile Flathead Catfish. It is estimated that maximum consumption for age-0 Flathead Catfish range from 33° C to 34° C while age-1 and older fish range between 31° C to 32° C (Roell and Orth 1993). Both maximum daily consumption and specific daily metabolic demand of juvenile Flathead Catfish increase with increasing temperatures (Bourret et al. 2008). Roell and Orth (1993) estimated the upper thermal limit of consumption for age-0 Flathead Catfish was 37° C while age-1 and older Flathead Catfish was 35° C. Bourret et al. (2008) reported consumption by Flathead Catfish ceased from 3° C to 7° C, was rare below 15° C, and significantly increased at 19° C and remained elevated up to 32° C.

The reliability of the parameters used in Flathead Catfish bioenergetics models are currently lacking. While some studies have used models to estimate food consumption and growth of Flathead Catfish (e.g., Roell and Orth 1993), biological parameters belonging to different species are often used instead. Such an approach is problematic, as parameters such as metabolism, growth, and temperature response are species-specific (Ney 1993; Trudel et al. 2004; Helfman et al. 2009).

Studying how temperature effects the physiological processes specific to juvenile Flathead Catfish provides managers with more reliable estimates of consumption, furthering insight into population dynamics of both native and non-native populations. My objectives for this project were to: 1) examine the effects of temperature on consumption of a single meal by juvenile Flathead Catfish (Chapter 2); 2) quantify evacuation rates of a single meal through the stomach and the intestines of juvenile Flathead Catfish (Chapter 2); and 3) determine the effects of temperature on absorption of a single meal by juvenile Flathead Catfish (Chapter 3).

LITERATURE CITED

Allan, B. J., P. Domenici, P. L. Munday, and M. I. McCormick. 2015. Feeling the heat: the effect of acute temperature changes on predator–prey interactions in coral reef fish. Conservation Physiology 3(1).

Andersen, N. G. 1999. The effects of predator size, temperature, and prey characteristics on gastric evacuation in Whiting. Journal of Fish Biology 54(2):287–301.

Boltaña, S., N. Sanhueza, A. Aguilar, C. Gallardo-Escarate, G. Arriagada, J. A. Valdes, D. Soto, and R. A. Quiñones. 2017. Influences of thermal environment on fish growth. Ecology and Evolution 7(17):6814–6825.

Başçınar, N., N. S. Başçınar, K. Seyhan, and U. Khan. 2016. The effect of temperature on the rate of gastric evacuation in Brook Trout *Salvelinus fontinalis* fed on commercial pellets: group-feeding. Pakistan Journal of Zoology 48(6):1899-1904.

Beitinger, T. L., and L. C. Fitzpatrick. 1979. Physiological and ecological correlates of preferred temperature in fish. American Zoologist 19(1):319–329.

Bourret, S. L., R. W. Tingley, Y. Kanno, and J. C. Vokoun. 2008. Maximum daily consumption and specific daily metabolic demand of juvenile Flathead Catfish *Pylodictis olivaris*. Journal of Freshwater Ecology 23(3):413–419.

Brett, J. R. 1956. Some principles in the thermal requirements of fishes. The Quarterly Review of Biology 31(2):75–87.

Brett, J. R. 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (Oncorhynchus nerkd). American Zoologist 11(1):99-113.

Brett, J. R. and T. D. D. Groves. 1979. Physiological energetics. Fish Physiology 8(6):280-352.

Bromley, P. J. 1988. Gastric digestion and evacuation in Whiting, *Merlangius merlangus*. Journal of Fish Biology 33(2):331–338.

Carmona-Catot, G., K. Magellan, and E. García-Berthou. 2013. Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. PLoS One 8(1).

Deslauriers, D., S. R. Chipps, J. E. Breck, J. A. Rice, and C. P. Madenjian. 2017. Fish bioenergetics 4.0: an R-based modeling application. Fisheries 42(11):586–596.

Dobbins, D. A., R. L. Cailteux, S. R. Midway, and E. H. Leone. 2012. Long-term impacts of introduced Flathead Catfish on native ictalurids in a north Florida, USA, river. Fisheries Management and Ecology 19(5):434–440.

Fry, F. E. J. 1971. The effect of environmental factors on animal activity. Pages 1–98. *In* W.S. Hoar and D.J. Randall, editors. Fish Physiology. Academic Press, New York.

Fuller, P. L., G. E. Whelan. 2018. The Flathead Catfish invasion of the Great Lakes. Journal of Great Lakes Research. 44(5): 1081-1092.

Fuller, P. L., L. G. Nico, J. D. Williams. 1999. Nonindigenous fishes introduced into inland waters of the United States. American Fisheries Society, Bethesda, Maryland.

Grigaltchik, V. S., A. J. Ward, and F. Seebacher. 2012. Thermal acclimation of interactions: differential responses to temperature change alter predator–prey relationship. Proceedings of the Royal Society B: Biological Sciences 279(1744): 4058-4064.

Gunn, D. L. 1942. Body temperature in poikilothermal animals. Biological Reviews 17(4): 293-314.

Handeland, S. O., A. K. Imsland, and S. O. Stefansson. 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic Salmon post-smolts. Aquaculture 283(1–4):36–42.

Hartman, K. J., S. B. and Brandt. 1995. Estimating energy density of fish. Transactions of the American Fisheries Society 124(3):347-355.

He, E., and W. A. Wurtsbaugh. 1993. An empirical model of gastric evacuation rates for fish and an analysis of digestion in piscivorous Brown Trout. Transactions of the American Fisheries Society 122:717–730.

Helfman, G. S., B. B. Collette, D. E. Facey, and B. W. Bowen. 2009. The diversity of fishes: biology, evolution, and ecology (2nd edition). Wiley-Blackwell, Chichester, U.K.

Hrabik, R.A., S.C. Schainost, R.H. Stasiak, and E.J. Peters. 2015. The fishes of Nebraska. Mennonite Press, Newton, Kansas.

Jackson, D. C. 1999. Flathead Catfish: biology, fisheries, and management. Catfish 2000: proceedings of the international ictalurid symposium. American Fisheries Society, Symposium 24, Bethesda, Maryland.

Jackson, D. A., P. R. Peres-Neto, and J. D. Olden. 2001. What controls who is where in freshwater fish communities – the roles of biotic, abiotic, and spatial factors. Canadian Journal of Fisheries and Aquatic Sciences 58(1):157–170.

Jobling, M. 1980. Gastric evacuation in Plaice, *Pleuronectes platessa*: effects of temperature and fish size. Journal of Fish Biology 17:547–551.

Jobling M. 1993. Bioenergetics: feed intake and energy partitioning. Pages 1-44 *in* J.C. Rankin and F.B. Jensen, editors. Fish Ecophysiology. Chapman and Hall Fish and Fisheries Series, Boundary Row, London.

Jobling, M. 1997. Temperature and growth: modulation of growth rate via temperature change. Pages 225–254 *in* C. M. Wood and D. G. McDonald, editors. Global Warming: Implications for Freshwater and Marine Fish. Cambridge University Press, Cambridge.

Jørgensen, C., K. Enberg, and M. Mangel. 2016. Modelling and interpreting fish bioenergetics: a role for behaviour, life-history traits and survival trade-offs: modelling and interpreting fish bioenergetics. Journal of Fish Biology 88(1):389–402.

Kapoor, B. G., H. Smit, I. A. Verighina. 1975. The alimentary canal and digestion in teleosts. Advances in Marine Biology. 13: 109-239.

Legler, N. D., T. B. Johnson, D. D. Heath, and S. A. Ludsin. 2010. Water temperature and prey size effects on the rate of digestion of larval and early juvenile fish. Transactions of the American Fisheries Society 139(3):868–875.

Minckley, W. L., and J. E. Deacon. 1959. Biology of the Flathead Catfish in Kansas. Transactions of the American Fisheries Society 88(4):344–355.

Molnar, C., and J. Gair. 2015. Concepts of Biology: 1st Canadian Edition. Available: https://openlibrary-repo.ecampusontario.ca/jspui/handle/123456789/345]

Moyle, P. B., and J. J. Cech. 2004. Fishes: an introduction to ichthyology. Pearson Prentice Hall, Upper Saddle River, New Jersey.

Nakagawa, H. 2018. Temperature-dependent gastric evacuation rate of the Japanese Delicate Loach *Niwaella delicata* (Cobitidae). Ichthyological Research 65(1):172–174.

Ney, J. J. 1993. Bioenergetics modeling today: growing pains on the cutting edge. Transactions of the American Fisheries Society 122(5):736–748.

O'Gorman, E. J., Ó. P. Ólafsson, B. O. L. Demars, N. Friberg, G. Guðbergsson, E. R. Hannesdóttir, M. C. Jackson, L. S. Johansson, Ó. B. McLaughlin, J. S. Ólafsson, G. Woodward, and G. M. Gíslason. 2016. Temperature effects on fish production across a natural thermal gradient. Global Change Biology 22(9):3206–3220.

Payne, N. L., J. A. Smith, D. E. van der Meulen, M. D. Taylor, Y. Y. Watanabe, A. Takahashi, T. A. Marzullo, C. A. Gray, G. Cadiou, and I. M. Suthers. 2016. Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. Functional Ecology 30(6):903–912.

Pedersen, T., and M. Jobling. 1989. Growth rates of large, sexually mature Cod *Gadus morhua*, in relation to condition and temperature during an annual cycle. Aquaculture 81(2):161-168.

Perrson, L. 1979. The effects of temperature and different food organisms on the rate of gastric evacuation in Perch *Perca fluviatilis*. Freshwater Biology 9:99–104.

Pflieger, W. L. 1997. The fishes of Missouri, revised edition. Missouri Department of Conservation, Jefferson City, Missouri.

Pine, W. E. III, T. J. Kwak, and J. A. Rice. 2007. Modeling management scenarios and the effects of an introduced apex predator on a coastal riverine fish community. Transactions of the American Fisheries Society 136(1):105-120.

Pine, W. E. III., T. J. Kwak, D. S. Waters, and J. A. Rice. 2005. Diet selectivity of introduced Flathead Catfish in coastal rivers. Transactions of the American Fisheries Society 134(4):901–909.

Roell, M. J., and D. J. Orth. 1993. Trophic basis of production of stream-dwelling Smallmouth Bass, Rock Bass, and Flathead Catfish in relation to invertebrate bait harvest. Transactions of the American Fisheries Society 122(1):46–62.

Seyhan, K., and D. J. Grove. 2003. A new approach in modelling gastric emptying in fish. Turkish Journal of Veterinary and Animal Sciences 27(5):1043–1047.

Smith, R. R. 1980. Nutritional bioenergetics in fish. *In* K. W. Chow, editor. Fish Feed Technology. FAO (Food and Agriculture Organization of the United Nations), ADCP/REP/80/11, Rome. Available: http://www.fao.org/3/x5738e/x5738e00.htm#Contents.

Somero, G. N. 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integrative and Comparative Biology 42(4):780–789.

Sweka, J. A., M. Keith Cox, and K. J. Hartman. 2004. Gastric evacuation rates of Brook Trout. Transactions of the American Fisheries Society 133(1):204–210.

Taniguchi, Y., F. J. Rahel, D. C. Novinger, and K. G. Gerow. 1998. Temperature mediation of competitive interactions among three fish species that replace each other along longitudinal stream gradients. Canadian Journal of Fisheries and Aquatic Sciences 55(8):1894-1901.

Trudel, M., D. R. Geist, and D. W. Welch. 2004. Modeling the oxygen consumption rates in Pacific Salmon and Steelhead: an assessment of current models and practices. Transactions of the American Fisheries Society 133(2):326–348.

Turner, D. R. 2017. Trophic dynamics of Flathead Catfish in the Missouri River bordering Nebraska. Master's thesis. University of Nebraska, Lincoln.

Van Zuiden, T. M., M. M. Chen, S. Stefanoff, L. Lopez, and S. Sharma. 2016. Projected impacts of climate change on three freshwater fishes and potential novel competitive interactions. Diversity and Distributions 22(5):603–614.

Viadero, R. C. 2005. Factors affecting fish growth and production. Water Encyclopedia 3:129-133.

Winberg, G. G. 1956. Rate of metabolism and food requirements of fishes. Fisheries Research Board of Canada, Translation Series 194, 1–253.

Windell, J. T., and D. O. Norris. 1969. Gastric digestion and evacuation in Rainbow Trout. The Progressive Fish-Culturist 31(1):20–26.

Zweifel, R. D., R. S. Hayward, and C. F. Rabeni. 1999. Bioenergetics insight into Black Bass distribution shifts in Ozark border region streams. North American Journal of Fisheries Management 19(1):192–197.

CHAPTER 2: THE EFFECTS OF TEMPERATURE ON GASTRIC EVACUATION RATES AND GUT PASSAGE TIME OF FLATHEAD CATFISH *PLYODICTIS OLIVARIS*

ABSTRACT

Knowledge of fish gastric evacuation rates are a necessary component for both field and laboratory studies to understand feeding rates, modeling energy budgets, and understanding trophic dynamics of aquatic ecosystems. Identifying the link between temperature and gastric evacuation rate is crucial to understand how the environment influences species-specific physiological processes. Freshwater species in temperate regions encounter a broad range of temperatures across life history stages, but the mechanistic link between temperature and metabolism often remains poorly understood, particularly for understudied species such as Flathead Catfish *Polydictis olivaris*. We examined the relationship between temperature and consumption, gastric evacuation rate, and meal passage through the intestines of juvenile Flathead Catfish fed a single ration. We ran 210 trials at three temperatures (17° C, 22° C, 25° C), of which 126 individuals consumed their ration. We dissected the digestive system of Flathead Catfish at 9 different time intervals post-consumption to examine passage of the ration at each temperature. Temperature significantly affected consumption of Flathead Catfish and analysis revealed significant differences in gastric evacuation rates between 17° C and 25° C and also 22° C and 25° C. We assessed the effect of ration size, Flathead Catfish mass, and digestion time on the proportion of the meal remaining

in both the stomach and the intestines. We found that digestion time, temperature, and ration size significantly affected the proportion of food remaining in the stomach. However, only digestion time and ration size significantly affected the rate of passage through the intestines, while temperature was not significant. Flathead Catfish are exposed to a wide breadth of temperatures on an annual basis (e.g., 0° C to 35° C in the Missouri River) and water temperatures are predicted to increase globally due to climate change. Insight into gastric evacuation rates provide managers with a better understanding of consumption demands and food passage of top predators.

INTRODUCTION

The field of physiology strives to incorporate a "sum of the parts" mentality when trying to understand the natural world (Horodysky et al. 2015). Environmental conditions influence the physiological performance of organisms, resulting in physiological and behavioral adjustments by individuals to acclimatize to new or changing conditions (Helmuth et al. 2005). We define physiology as the study of how the body of an animal functions and responds to its environment. Considerable focus on the physiological processes of fish has been given to a few species, namely that of salmonids among others (Eddy and Handy 2012), while the vast majority remain poorly understood.

Researchers cannot accurately rely on highly studied species to explain the physiological processes occurring within understudied species. Similarly, a single

life-history stage cannot accurately explain the functions and processes occurring throughout the life of a species. This is due to unique energetic, physiological, and behavioral processes based on life-history characteristics of a species and their environment. For example, Whitledge et al. (2010) found that error in model predictions were reduced when using physiological parameters of a species, rather than borrowing these parameters. To increase accuracy of predictive models (e.g., models of growth and food consumption), there is a need to understand physiological processes of poorly understood species.

Fish digestion has received considerable attention from fish physiologists, dating back to the 1930's (reviewed by Kapoor et al. 1975). Laboratory experiments have been a crucial component contributing to knowledge of fish physiology of the gut and the overall digestive process (Talbot 1985). To understand the energy flow through a fish, we first have to examine the rate at which a ration is processed along the digestive system. Fisheries biologists typically study the rate at which the stomach empties after consuming a ration, known as the gastric evacuation rate. Studies examining gastric evacuation rates assume that over longer periods of time the rate at which food is evacuated from the stomach is equal to the rate at which food is being consumed (Seyhan and Grove 2003).

Understanding gastric evacuation rates are necessary in both aquaculture and management of natural aquatic systems when trying to understand feeding rates, energy budgets, and trophic dynamics of a system (Sweka et al. 2011).

Species-specific temperature-dependent optimal feeding rates are well established in aquaculture for commonly used species. Underfeeding fish can lead to poor growth, while overfeeding fish can lead to poor water quality, decreased absorption efficiency, and wasting of food resources (Ndome et al. 2011; Başçınar et al. 2016; Craig et al. 2017). Understanding the food intake of a given population within its natural habitat is also of interest as gastric evacuation experiments have been used to quantify consumption rates of natural fish populations and determine the impacts on prey populations (Elliott and Persson 1978, Bromley 1994; Seyhan et al. 1998).

Several methods have been used to study gastric evacuation including serial slaughter (Başçınar et al. 2016), gastric lavage (Sweka et al. 2004; Waters et al. 2004), among others (reviewed by Langton 1977). Multiple variables play a role in gastric evacuation (Bromley 1987; He and Wurtsbaugh 1993) including fish size (Andersen 1999), type of prey item (Windell and Norris 1969), prey size (Legler et al. 2010), and temperature (Perrson 1979; Jobling 1980; Nakagawa 2018). Temperature is thought to be the most influential factor regulating gastric evacuation rates since temperature can influence the digestive process in a variety of ways including feeding rates, secretion rates of digestive fluids and enzymes, gastric and intestinal motility (Kapoor et al. 1975). Multiple studies have reported that an increase in temperature results in an increase in gastric evacuation rate up to a threshold before diminishing (Tyler 1970; Bernreuther et al. 2009; Andersen 2012).

Flathead Catfish *Plyodictis olivaris* are an endemic North American fish ranging west of the Appalachian Mountains throughout the Mississippi River Basin (Hrabik et al. 2015), as well as the Mobile and Rio Grande River Basins. Flathead Catfish distribution highlights the thermal plasticity of this species, which can withstand a wide range of temperature regimes both spatially and seasonally. Despite Flathead Catfish widespread distribution and major concerns of introductions outside their native ranges (e.g., Fuller and Whelan 2018; Brown et al. 2005), few studies have examined their gastric evacuation rates. Fewer studies have evaluated how specific environmental and ecological variables may influence digestive processes in this species.

Understanding the specific link between temperature and gastric evacuation for juvenile Flathead Catfish provides managers with knowledge of fish consumption during a crucial life stage. Together, consumption and gastric evacuation rates begin to provide insight on fish growth. During the juvenile life-history stage, production is prioritized with somatic growth of the body and the functioning systems that sustain life of the individual (Wootton and Smith 2015). Eventually, adulthood results in a more complex allocation of energy toward reproduction at a cost to somatic growth (Rijnsdorp 1990; Wootton and Smith 2015).

The accuracy of bioenergetics predictions varies greatly across seasons for various species (Minton and McLean 1982; Chipps et al. 2000). Water temperature is influenced by a multitude of natural (e.g., air temperature and

runoff rates) and anthropogenic factors (e.g., water diversion. reservoir release, and thermal discharge: reviewed by Caissie 2006), which will inevitably alter consumption and gastric evacuation rates. Our objective was to address the knowledge gap of temperature interacting with consumption and evacuation rates of juvenile Flathead Catfish. We predicted that increased temperatures would result in increased consumption, gastric evacuation rate, and passage of the meal through the intestines.

METHODS

Fish collection

Collection of Flathead Catfish took place in the fall of 2018. Flathead Catfish were collected from the Missouri River near Blair, Nebraska, U.S.A. between river mile 660 and 645. Flathead Catfish were sampled using a 15 Hz, 3-4 amp pulsed DC electric field produced by a generator (Kohler, Kohler, Wisconsin, U.S.A.) and a 5.0 GPP electofisher control box (Smith-Root, Vancouver, Washington, U.S.A.) mounted on a boat. We targeted Flathead Catfish ranging from 200 mm to 400 mm in total length. Maximum size of 400 mm was enforced to reduce the chances of the Flathead Catfish being reproductively mature. Targeting a specific size range was intended to reduce the variability of diet (e.g., ontogenetic dietary shifts) and deter from energy being allocated toward reproductive growth of the individual. Flathead Catfish were held in a live well during collection and were transported via aerated coolers back

to the University of Nebraska – Lincoln Fish Conservation, Behavior, and Physiology Laboratory.

Acclimation and rearing tanks

Flathead Catfish (n = 210) were randomly divided into three treatment (temperature) groups. System 1 was set to 17° C, system 2 was set to 22° C, and system 3 was set to 25° C. Temperatures were maintained within ±1° C of the desired rearing system temperature. Temperatures were selected based on past studies, typical field conditions, and laboratory limitations. Bourret et al. (2008) determined that Flathead Catfish rarely ate below 15° C, while maximum daily consumption was significantly higher at 19° C and remained elevated until 32° C. Furthermore, yearly Missouri River temperatures that Flathead Catfish are found in Nebraska range between 15° C to 30° C from May to October, peaking in mid-July (USGS 2019).

Acclimation included a 12-hour light, 12-hour dark photoperiod, with lights coming on at 0545 and turning off at 1745. Flathead Catfish were fed Fathead Minnows *Pimephales promelas* sourced from a commercial vendor (Rainbow Bait, Ruthton, Minnesota, U.S.A.). We chose Fathead Minnows based on availability. Flathead Catfish fed freely within each rearing tank and we replenished consumed Fathead Minnows daily, ensuring food was always present.

Recirculating aquatic system underwent daily formalin treatments conducted at 125 ppm to kill *Ichthyophthirius multifiliis* (Ich) and *Trichomonas*

gallinae (Trich) brought into the laboratory by wild fish. Treatments were conducted for a minimum of 1-week in all systems. Water temperatures below 25° C required an additional week of treatment due to the life cycle of the parasites. Random skin scrapes were used to ensure the eradication of Ich and Trich. Fathead Minnows underwent a similar formalin quarantine prior to being placed in a recirculating aquatic system as food for Flathead Catfish.

Daily log books were kept for temperature and dissolved oxygen using a YSI Pro 20 (YSI Inc., Yellow Springs, Ohio, U.S.A.) for each of the rearing and experimental tanks. We collected weekly data on ammonia, nitrite, nitrate, pH, and total chlorine levels of each system. Weekly backwashing of the sand filter accompanied by 25% water changes every other day were conducted to reduce the buildup of nitrate within the systems. Experimental trials were initiated after a three-week acclimation period to laboratory conditions.

Each system consisted of three large, 1.22 m diameter, 757 L in-line rearing tanks, and one large, 1.22 m diameter, 757 L in-line experimental tank (Figure 2-1; See Appendix 1 for details on rearing Flathead Catfish in a recirculating aquatic system). Tanks A and B held most of the sample subjects (Figure 2-2). Tank C held a sub-sample of tagged Flathead Catfish undergoing a gut microbiota experiment for the first 6 weeks of their introduction into the laboratory. Once the microbiota experiment had concluded these fish were tested in our trials after a re-acclimation period of three weeks of no handling. Tank D was reserved for experimental aquaria set up.

Experimental tank set up

Two experimental aquaria built from PVC and shade cloth were placed into tank D of each system (Figure 2-3). Water passed through the constructed tanks freely but individuals placed within the tanks were unable to see one another. Experimental aquaria within tank D were elevated by cinderblocks to ensure that the fish could not escape into the surrounding tank. Tank D was in-line with the rest of the rearing system. Therefore, conditions such as water quality and temperature did not differ from conditions that fish were acclimated to. A shade cloth canopy over tank D was used to minimize stress and experimenter influence. A surveillance camera (Zosi HD 720p, Zosi Technology, Hong Kong, China) was mounted above each experimental aquarium to record feeding times and behavior. Cameras recorded video both during the daytime and nighttime hours, and video was stored on a DVR with 1TB of storage.

Experimental procedure

Rearing tanks were placed on rotation of a 24-hour fasting period. Flathead Catfish were randomly selected from a fasted rearing tank, placed into an experimental aquarium, and fasted an additional 24 hours. We assumed that fasted individuals of 48 hours had empty digestive tracts prior to introducing a food source. Furthermore, we assumed introduced food would be consumed quickly. Previous research has shown that fish gastric evacuation rates are not

influenced by starvation periods between 1 to 5 days (Elliott 1972); therefore, we assumed that a 5-day starvation period had no effect on gastric evacuation rates.

Flathead Catfish were fed a single ration of Goldfish *Carassius auratus* (e.g., one individual Goldfish) measured to the nearest 0.01 g. Goldfish health was assessed daily and were replaced with healthy individuals if needed. Goldfish were used in the experimental aquaria because they provided more mass per consumption event than a single Fathead Minnow and would be easier to test. Goldfish were also easier to see in experimental aquaria via cameras.

Flathead Catfish were given 72 hours to consume a Goldfish. At 72 hours post introduction of a Goldfish, Flathead Catfish were known to have not eaten for a total of five days. Flathead Catfish that did not eat during the 72-hour period were removed from our experiment and no data was collected from the individual. Flathead Catfish were only tested one time.

Camera footage was reviewed throughout the day to identify if consumption took place. Time of consumption was recorded and time of euthanasia was set based on one of nine predetermined time intervals (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h). Setting euthanasia and dissection times from the point at which Flathead Catfish consumed a Goldfish provided a known amount of time to pass for digestion to occur. Therefore, we use dissection and digestion time interchangeably. Digestion time intervals were selected based on previous gastric evacuation rate studies (e.g., Ling and Ghaffar 2014). Our goal was to collect data on 6 individuals at each digestion time for each treatment (n = 54).

Flathead Catfish that consumed a Goldfish were euthanized in a buffered tricaine mesylate (MS-222) solution prior to dissection.

Data collection

Euthanized Flathead Catfish were measured to the nearest 1.0 mm (total length) and 0.01 g. Flathead Catfish were dissected immediately following euthanasia and the entire digestive tract was removed, esophagus to the anus. We divided the digestive tract in to two sections, the stomach and intestines (Figure 2-4). Digested contents were extracted by gently squeezing along the stomach and the intestines. Dissection scissors were used to cut open the stomach and intestines to ensure all the contents were collected. Digested matter was weighed to the nearest .01 g (wet weight) and saved for bomb calorimetry analysis (Chapter 3 of this thesis). Vials containing digested content were placed in a -80° C freezer until all trials were completed.

We sexed Flathead Catfish during dissection. If gonad development was distinguishable the gonads were removed and measured to the nearest 0.01 g (wet weight. Additionally, we collected pectoral spines as an aging structure.

Validation of Flathead Catfish as juveniles

Physiological processes are known to differ among life stages of fishes (e.g., Deslauriers 2017) and as male and female fish mature they may exhibit differences in evacuation rates due to physiological demands or constraints.

Therefore, it was important for us to establish a definition of a juvenile Flathead Catfish. We defined juvenile Flathead Catfish as individuals that are non-reproductive (Wootton and Smith 2015). We consider this to include individuals where sexual differentiation has occurred and fish are undergoing puberty, a sub-adult stage. We defined puberty as the transitional phase between a non-reproductive juvenile and a sexually mature adult (Wootton and Smith 2015). Although our definition is subjective, it provides a clear cut off for when we considered juvenile Flathead Catfish to transition into adult Flathead Catfish. Such a definition provides a reference for future studies in comparing consumption and evacuation rates of juvenile Flathead Catfish.

We determined ages of dissected Flathead Catfish using sectioned pectoral spines (methods similar to Buckmeier et al. 2002). Spines were removed from Flathead Catfish after gastric evacuation rate data collection was complete and allowed to dry in coin envelopes. Pectoral spines were set in molding clay and encased by a hollow vial that was then filled with a clear epoxy. Three sections around the basal recess, approximately 30 μm thick, were cut with a low-speed isomet saw. Crosscut sections were mounted to a slide and examined under a microscope. Yearly annuli rings were counted in a similar method of aging catfish developed by Sneed (1951).

We established a threshold for sexual maturity in Flathead Catfish using the gonadosomatic index (GSI). The gonadosomatic index measures gonad mass as a proportion to body mass and is calculated using the equation:

$$GSI\ \% = \frac{Weight\ of\ gonads\ (g)}{Weight\ of\ fish\ (g)} * 100.$$

We used a threshold of 0.36 % to define a mature male and 6.5 % to define a mature female based on a study examining sexual maturity of Channel Catfish *Ictalurus punctatus* (Mahoney 1982). Gonadosomatic index values were calculated for all individuals that we were able to differentiate male and female gonad development. Individuals with a gonadosomatic index value greater than the established threshold were removed from analysis.

We compared age, sex, and gonadosomatic index values among the three treatments to determine if juveniles were randomly distributed using a one-way ANOVA. Additionally, we created a generalized linear model to examine whether age, sex, and treatment had significant effects on gonadosomatic index values. Post-hoc analysis were ran to determine differences between age groups.

Water quality and ration size analysis

We examined water quality parameters (e.g., temperature, dissolved oxygen, ammonia, nitrite, nitrate and pH) for homogeneity within each system and among treatments. Additionally, weights (both Flathead Catfish and Goldfish) and ration sizes (meal weight as a proportion of Flathead Catfish body weight) were examined to ensure measures were randomly distributed among treatments. We conducted ANOVA tests in order to validate our experimental design and our ability to successfully control for variables that could confound evacuation rate results.

Data analysis for consumption

Generalized linear models were used to evaluate the effects of prey weight, temperature, age, sex, gonadosomatic index value, and experimental tank on consumption. Consumption was quantified as a binomial by issuing a "1" for fish that consumed a Goldfish and a "0" for fish that did not (Zar 2010). We used the corrected Akaike's Information Criterion (AICc) to assess which generalized linear model was our best-fit model. If multiple models were within 2.0 delta AICc from the best-fit model, we chose the simplest model (e.g., the model with the fewest parameters; K). A Chisquare test was used to determine which terms within our best-fit model were significant. We used least square means for multiple comparisons with a Tukey-adjustment of p-values (lsmeans package in R) to test differences among treatments (Lenth and Hervé 2015).

Data analysis for gastric evacuation rates

Digestion time, temperature, predator size, prey size, and ration size are known to effect gastric evacuation rates of fishes. We assumed that sex, experimental tank, and feeding time could also be influencing gastric evacuation rates of our sample. Prey wet weights have been found to be significantly correlated with dry weights of prey species (Glenn and Ward 1968); therefore, we used wet weights for gastric evacuation rate analysis.

We standardized the dependent variable among individuals by calculating the proportion of the meal remaining in the stomach by dividing the weight of the stomach contents by the initial weight of the Goldfish fed to the Flathead Catfish. We assumed the proportion of the meal remaining in the stomach would equal 1.0 at time of consumption followed by a horizontal asymptote near 0.0 sometime after consumption. We ran generalized linear models (family = binomial) on the proportion of the meal remaining in the stomach on the aforementioned independent variables.

AICc was used to narrow down the best-fit model. If multiple models fell within 2.0 delta AICc of the best-fit model, we chose the simplest model. A Chisquare test was used to determine which terms within our best-fit model were significant. While ration sizes are likely to vary in the wild, the primary focus of this paper was to focus on the influences of temperature and therefore whenever ration size was a significant model predictor, it was held constant with its mean value of 0.015. Least square means for multiple comparison (using Tukey-adjusted comparisons) was used to determine differences among treatments.

We created a predictive model for gastric evacuation rates based on our best-fit model. Binomial regression output is in units of logits, which can be transformed into odds with:

$$odds = \exp(logits)$$

and then a proportion with:

$$proportion = odds/(1 + odds).$$

Using this model, we predicted gastric evacuation rates (instantaneous slope of the regression) and gastric emptying times of juvenile Flathead Catfish among our treatments. Gastric emptying time provides an estimate of how long it takes for the stomach to empty or pass the meal into the intestines. During the later stages of digestion within the stomach, gastric evacuation rates become highly variable and decrease (Windell 1966; Magnuson 1969; Tyler 1970), therefore values of 90% depletion may be more valuable and accurate in determination of daily ration calculations (Swenson and Smith 1973). Under this assumption, we considered a proportion of 0.10 food remaining in the stomach to be "empty."

Data analysis for intestinal passage

We examined the effect of temperature on the passage of food through the intestines of Flathead Catfish using generalized linear models. We calculated the proportion of the meal remaining in the intestines by dividing the weight of intestinal contents at time of dissection by the initial weight of the Goldfish fed to the Flathead Catfish. We examined the same independent variables tested in the proportion of the meal remaining in the stomach. However, in modeling the intestines, we incorporated a quadratic function to capture the parabola vertex of the ration entering and exiting the intestines. Unlike the stomach, where the proportion of the meal was assumed to be 1.0 at time of consumption and diminished through time, the intestines were assumed to be 0.0 at the time of

consumption and show a gradual increase in contents followed by a peak and decrease back to 0.0.

Photoperiod cues on feeding

Empirically derived research is limited in examining the feeding behavior of Flathead Catfish, particularly whether they are diurnal or nocturnal feeders. Our laboratory lights were on an automated system, on for 12 hours and off for 12 hours, with no windows or natural lighting. We were interested in whether 'day' or 'night' conditions influence the occurrence of consumption. We created a dummy variable to explain whether lights were on (1) or off (0) at the time of consumption. We used a Poisson regression analysis because outcome variables were represented as counts (Zar 2010). An empirical cumulative distribution function (ECDF) was used to illustrate the percent of the observed total Flathead Catfish to consume a Goldfish over a 24 hour day. A high occurrence in the number of feedings results in a steeper slope of the ECDF. This behavioral analysis is useful to understanding temporal feeding habits of Flathead Catfish.

RESULTS

Summary statistics

A total of 210 trials were conducted among the three treatments, with 126 consumption events and 84 non-consumption events. The mean total length for dissected Flathead Catfish was 300 mm (SD = 50 mm) and a mean weight of 255

g (SD = 125 g). Goldfish had a mean weight of 3.00 g (SD = 1.07 g), which resulted in a mean ration size of 1.51 % (SD = 1.01 %). We identified 55 males and 52 females during dissection, and could not identify the sexes of 19 Flathead Catfish (Table 2-1).

System 1 (mean temperature = 17.0º C; SD = 0.5º C) had a total of 77 trials conducted with 33 Flathead Catfish consuming a Goldfish (43%). The average total length of Flathead Catfish within system 1 was 301 mm (SD = 47 mm) and had an average weight of 259 g (SD = 105). The average ration size was 1.40% (SD = 1.01%) of the body weight of the Flathead Catfish. There were a total of 11 males, 19 females, and 3 unknown Flathead Catfish (Table 2A-1).

System 2 (mean temperature = 21.9º C; SD = 0.6º C) had a total of 65 trials conducted with 38 Flathead Catfish consuming a Goldfish (58%). The average total length of Flathead Catfish within system 2 was 287 mm (SD = 58 mm) and had an average weight of 240 g (SD = 141 g). The average ration size was 1.70% (SD = 1.24%) of the body weight of the Flathead Catfish. There were a total of 18 males, 14 females, and 6 unknown Flathead Catfish (Table 2A-2).

System 3 (mean temperature = 24.8º C; SD = 0.4º C) had a total of 68 trials conducted with 55 Flathead Catfish consuming a Goldfish (81%). The average total length of Flathead Catfish within system 3 was 306 mm (SD = 42 mm) and had an average weight of 262 g (SD = 127 g). The average ration size was 1.44% (SD = 0.83%) of the body weight of the Flathead Catfish. There were a total of 26 males, 19 females, and 10 unknown Flathead Catfish (Table 2A-3).

Validation of Flathead Catfish as juveniles

Age of Flathead Catfish ranged from 1 to 5 years old (mean = 2.23 years; SD = 0.76 years) and did not differ among treatment groups ($F_{2,120} = 0.956$, P = 0.387). Determination of age alone did not establish whether Flathead Catfish were juveniles based on our definition. Therefore, we calculated gonadosomatic index values for Flathead Catfish that we were able to differentiate gonads as male or female. Sex of Flathead Catfish did not differ among treatments ($F_{2,104} = 1.832$, P = 0.165). All gonadosomatic index values were below the established threshold for sexual maturity, ranging from 0.03 % to 0.29 % for males and 0.11 % to 1.36 % for females (Table 2A-4). Gonadosomatic index values differed between sex (P < 0.001) and among age (P < 0.01), but not among treatments (P = 0.178, Table 2-2). Results suggest that Flathead Catfish were appropriately labeled as juveniles based on our definition, and juveniles were randomly distributed among treatments. No individuals were removed from our analysis due to being classified as an adult. A post-hoc analysis found differences between ages 1 and 3 (P = 0.004), 1 and 5 (P < 0.001), 2 and 5 (P < 0.001), 3 and 5 (P < 0.001), and 4 and 5 (P = 0.001; Table 2A-5).

Water quality

Temperature among tanks of each system (e.g., four tanks per system) did not differ (P > 0.05; Figure 2-5) while dissolved oxygen levels were different (P <

0.05; Figure 2-6). A post-hoc analysis resulted in differences in dissolved oxygen levels among tanks A-B ($P < 0.001$), A-C ($P < 0.001$), A-D ($P = 0.011$), and C-D ($P = 0.047$) in system 1; difference in dissolved oxygen levels among tanks A-C ($P < 0.001$) and C-D ($P = 0.002$) of system 2; and no difference in dissolved oxygen levels among tanks of system 3. Differences of dissolved oxygen levels among tanks could be due to sensitivity of the YSI probe and we do not believe these differences would have a biologically meaningful effect on consumption and evacuation rates within our study. Ammonia ($F_{2,57} = 0.364$, $P = 0.697$), nitrite ($F_{2,57} = 0.961$, $P = 0.388$), nitrate ($F_{2,57} = 0.029$, $P = 0.971$), and pH ($F_{2,57} = 0.542$, $P = 0.585$) did not differ among the three treatments (Table 2-3) from the time of acclimation to the conclusion of the experiment (September 24, 2018 through February 4, 2019). We did not experience any natural mortalities of Flathead Catfish.

Predator and prey weights

Wet weight of Flathead Catfish that consumed a ration were not different ($F_{2,123} = 0.374$, $P = 0.689$, Figure 2-7) among treatments. Additionally, the wet weight of Goldfish consumed by Flathead Catfish were not different ($F_{2,123} = 0.283$, $P = 0.754$, Figure 2-8) among treatments. We calculated ration size by dividing Goldfish weight from the Flathead Catfish weight to standardize meal sizes among all individuals. We found that ration size ($F_{2,123} = 0.945$, $P = 0.392$, Figure 2-9) was not different among treatments.

Consumption

Occurrences of consumption increased as temperature increased (Figure 2-10). Two models (Table 2-4) showed support in prediction of consumption. Model 1 accounted for temperature as a main effect. Model 2 accounted for prey weight and temperature as main effects. Temperature was significant in both models, but adding prey weight did not significantly improve the model (P = 0.087, Table 2-5). Therefore, we used the simplest model of temperature as the predictor of consumption. There was a significant difference (χ^2 = 22.84, df= 2, P < .001) in consumption among treatments. Results from least square means comparison showed a difference in consumption between treatments of 17º C and 25º C (P < 0.001) and 22º C and 25º C (P = 0.021), but not between 17º C and 22º C (P = 0.127; Table 2-6).

Gastric evacuation

The mean proportion of the meal remaining in the stomach decreased as digestion time increased across all three treatments (Table 2A-7). The proportion of the meal remaining in the stomach was best predicted by digestion time, temperature, and ration size (Table 2-7). Digestion time (χ^2 = 205.94, df= 1, P < 0.001), temperature (χ^2 = 12.10, df= 2, P = 0.003), and ration size (χ^2 = 8.25, df= 1, P = 0.004) were all significant predictors within the model (Table 2-8). A post-hoc analysis using least square means for multiple comparison found differences

in gastric evacuation rates between treatments of 17° C and 25° C (P = 0.002), but not 17° C and 22° C (P = 0.076), or 22° C and 25° C (P = 0.490; Table 2-9).

We ran a binomial logistic regression on the proportion of the meal remaining in the stomachs of juvenile Flathead Catfish (Figure 2-11). Our logistic regression equation was:

$$PropRemainS` = 2.282 - 0.257(t) - 0.901(T_{22}) - 1.303(T_{25}) + 38.461(Rs),$$

where *PropRemainS`* = predicted logits value of the meal remaining in the stomach; *t* = digestion time; T_{22} = binary input (0 or 1) used to examine 22° C fish; T_{25} = binary input (0 or 1) used to examine 25° C; and *Rs* = ration size. For example, when estimating the proportion of the meal remaining in the stomach of a 17° C fish (the reference group), T_{22} and T_{25} = 0. When estimating the proportion of the meal remaining in the stomach of a 22° C fish, T_{22} = 1 and T_{25} = 0. When estimating the proportion of the meal remaining in the stomach of a 25° C fish, T_{22} = 0 and T_{25} = 1.

Examining 2 hours post consumption, the predicted proportion of the meal remaining in the stomach was 0.91, 0.81, and 0.74 for 17° C, 22° C and 25° C fish. At 12 hours post consumption, the predicted proportion of the meal remaining in the stomach was 0.44, 0.24, and 0.18 for 17° C, 22° C and 25° C fish. Our model predicted the stomach of juvenile Flathead Catfish was empty at 19 hours 39 minutes, 16 hours 9 minutes, and 14 hours 33 minutes for 17° C, 22° C, and 25° C fish (Table 2-10).

Passage of intestinal contents

We determined that digestion time, ration size, and a fitted parabola function (digestion time^2) were the best predictors of the proportion of the meal remaining within the intestines (Table 2-11). Two additional models had support but we chose not to use them based on the significance of the model predictors and the number of model parameters. Digestion time (P = 0.029) and ration size (P = 0.008) were significant predictors within the best-fit model, and the fitted parabola function approached significance (P = 0.053; Table 2-12).

Temperature was not a predictor variable within our best-fit model; therefore, we combined all three treatments to run a binomial logistic regression on proportion of the meal remaining in the intestines (Figure 2-12). Our logistic regression equation was:

$$PropRemainI` = -0.7889 + 0.0474(t) - 0.0014(t^2) - 34.3211(Rs),$$

where PropRemainI` = predicted logits value of the meal remaining in the intestines, t = digestion time, t^2 = digestion time^2, Rs = ration size.

Photoperiod cues on feeding behavior

We combined the number of Flathead Catfish to consume a Goldfish to one-hour time intervals over a 24-hour day (Table 2A-8). On average, 8.0 (SD = 7.0) Flathead Catfish consumed a Goldfish per hour when the lights were off in the laboratory. Conversely, 3.0 (SD = 2) Flathead Catfish consumed a Goldfish

per hour lights were on in the laboratory (Figure 2-13). There was a significant difference ($F_{1,22} = 6.619$, $P = 0.017$) between our light status groups (Table 2-13). With the lights off, 98 (77%) Flathead Catfish consumed a Goldfish, while only 28 (22%) Flathead Catfish consumed a Goldfish when lights were on in the laboratory (Figure 2-14). Furthermore, 38% of consumption occurred between 1745 (lights off) and midnight, 36% of consumption occurred between midnight and 0545 when the lights came back on in the laboratory, and 25% of consumption occurred between 0545 and 1745 (lights on). Additionally, 19% of total consumption occurred within 20 minutes after the light were shut off in the laboratory (Figure 2-15).

DISCUSSION

The effect of temperature on consumption

Our results showed that temperature significantly influenced consumption of Goldfish by juvenile Flathead Catfish. As temperature increased among treatments the number of observed feedings also increased (43% at 17° C, 59% at 22° C, and 81% at 25° C), supporting research from Bourret et al. (2008) that found specific daily consumption of juvenile Flathead Catfish increased with increasing temperatures. Our results showed a significant increase in consumption at 22° C from 17° C in general agreement with Bourret et al. (2008) who reported a significant increase in consumption at 19° C. We suspect that increasing

temperatures led to an increase in consumption to keep up with increased metabolic demands at warmer temperatures.

Effect of temperature on gastric evacuation

We found that temperature, ration size, and digestion time had an effect on the proportion of the meal remaining within the stomachs of juvenile Flathead Catfish. Ration size has been found to be influential in gastric evacuation rates of other fishes (Sweka et al. 2004), though this can vary between and within species. Our data suggests that at 19 and 14 hours post consumption, approximately 10% of the meal remained in the stomach at 17º C and 25º C, respectively. Extrapolating our results to the population level would suggest that prey biomass necessary to support consumption rates of juvenile Flathead Catfish would need to increase as temperature increases, based on our results of quicker gastric evacuation times at warmer temperatures. This is in large part due to increased metabolic demand and appetite returning earlier for warmer water individuals. Appetite-regulating peptides in the stomachs of fishes have various responses (increasing or suppressing) that control hunger (Rønnestad et al. 2017). Research by Grove et al. (1978) reported Rainbow Trout *Salmo gairdneri* appetite returned when 80-90% of stomach content had emptied. Quicker gastric evacuation rates at higher temperatures result in a faster return of appetite as explored in other studies (e.g., Sims et al. 1996; Riche et al. 2004). Our results suggest that increasing gastric evacuation rates, as a response of increasing temperature, increase the

occurrence of consumption by juvenile Flathead Catfish. It is useful for biologists to understand the mechanistic link between temperature, consumption, and gastric evacuation rates at the individual level to better manage a population. In studies where estimates of food consumption by fish in the field are impractical, laboratory derived gastric evacuation rates can be used with estimates of stomach fullness from the field to predict food consumption rates (Swenson and Smith 1973). Previous field studies have attempted to quantify consumption rates of Flathead Catfish populations based on field estimates of gastric evacuation rates and stomach fullness (e.g., Turner 2017). Such estimates are useful but only provide snapshot of consumption rate occurring at that particular time. Extrapolating consumption rates beyond the water temperatures within the field at time of sampling can be problematic. Our results suggest that gastric evacuation rates of juvenile Flathead Catfish are different based on water temperature and will vary among seasons, ultimately influencing estimates of consumption rates throughout the year.

Our study begins to broaden our understanding that warmer temperatures increase the gastric evacuation rates of juvenile Flathead Catfish, which then increase consumption rates of individuals. We selected three treatment temperatures based on a native thermal regime that juvenile Flathead Catfish may experience. We were limited in our ability to examine temperatures at the predicted upper thermal range of juvenile Flathead Catfish. Gammon (1973) reports that the optimum temperature (for growth, swimming speed, and “a

variety of physiological parameters") for Flathead Catfish ranges from 31.5-33°C. Roell and Orth (1993) predicted temperatures of 35° C as the upper avoidance temperature for juvenile Flathead Catfish. Future studies examining gastric evacuation rates of juvenile Flathead Catfish would benefit exploring warmer temperatures (>25° C), as the thermal environment of Flathead Catfish range beyond what we were able to examine. Such findings would be beneficial in broadening biologists understanding of juvenile Flathead Catfish consumption rates at a wider geographic range.

Increased knowledge of the effect of temperature on consumption and gastric evacuation rates is needed to better understand growth rates. Flathead Catfish have large growth potential (Tetzlaff et al. 2010), and likely high consumption rates (Roell and Orth 1993) across a wide range of temperatures. We found that temperature influenced consumption at a relatively narrow range (i.e., a change of 8° C), indicating that juvenile Flathead Catfish are sensitive to changes in temperature. This sensitivity to temperature could be increasing consumption at lower temperatures, potentially allowing Flatheads to outcompete other fish species such as Channel Catfish. Flathead Catfish in the wild are exposed to a wide breadth of temperatures and given their highly plastic nature, a large range of temperatures may be suitable for growth, allowing this species to continue consuming and growing when its competitors are not.

The influence of temperature on intestinal passage

While most studies are primarily concerned with gastric evacuation rates to better understand daily consumption of a species, little is known about the role of temperature on passage of a meal through the intestines. Understanding the physical breakdown of food provides insight into meal passage in the intestines. Digestion within the stomach occurs as a gradual inward process where layers of the consumed meal are reduced and transported to the intestines (Kapoor et al. 1975). Mucus, acid, and enzymes within the stomach break down the solid state of the food forming a liquid as it passes through the midgut before it solidifies forming feces in the hindgut (Smith 1980). Nearly all biological rates depend strongly on temperature (Brown et al. 2004); however, our experiment found that temperature was not significant in explaining passage of the meal through the intestines. It is likely that the majority of food passed into the intestines is at a stage of digestion similar across temperatures and likely explains why the rate of passage was similar among our treatments.

Our model predicts the proportion of the meal remaining across all treatment temperatures for any given time point. We predicted that 23% of the initial meal was present within the intestines 2 hours post-consumption. This prediction supports Kapoor et al. (1975), which suggested that digestion occurs in layers where easy to digest contents are quickly passed from the stomach to the intestines. Furthermore, we predicted that intestinal content peaked at 29% of the initial meal fed to juvenile Flathead Catfish around 16 hours post consumption. These results are supported by our gastric evacuation rate models that predicted

gastric emptying times ranged from 14 to 19 hours, which corresponds with maximum intestinal content. For example, the moment we considered the stomach empty, no more content would be entering the intestines and it is at the fullest it will be prior to waste being expelled.

At 48 hours post consumption, our model predicted that 9% of the initial meal would remain within the intestines. Our results show that none of our 17º C fish at the 48-hour period (n = 4) had completely empty intestines (approximately 20% of the initial meal remained). However, we began to observe certain individuals at 22º C and 25º C drop to 0% intestinal content remaining at 48 hours post consumption, creating a noticeable gap in the percent of the meal remaining in the intestines among individuals. Our results could suggest that feces begin to pass in large clumps and not at a steady declining rate around this 48-hour time period. This could result in large potential losses of energy from a meal being absorbed. For example, Brett and Groves (1979) report that 20-40% of energy of a meal is lost through feces. Therefore, it would be beneficial to examine the effect of temperature on energy remaining in a meal through the digestive tract of juvenile Flathead Catfish.

Lighting cue on feeding behavior

We found strong evidence that feeding behavior was cued by photoperiod with elevated consumption occurring during the nighttime hours (between 1745 and 0545). No fish were consumed between 0600 to 0700, but this may be due to

experimental design, in which we typically introduced food between the hours of 0800 and 1100. Some fish were likely more aggressive feeders and consumed their prey upon introduction while others waited until darkness within the laboratory, or until the second day and evening to feed. Our study supported the results of Minckley and Deacon (1959) and Quinn (1988), who reported Flathead Catfish showing crepuscular and nocturnal patterns of feeding.

Biologists interested in collecting stomach contents of Flathead Catfish benefit in understanding the behavioral cue lighting has on feeding. Our results suggest that the time at which sampling takes place in the field could be inflating empty stomach numbers or stomach fullness. Based on our results, and under the assumption that gastric evacuation rates of different stomach fullness proceed at the same rate, if the water temperatures were warm (>25º C) and a majority of the meal was consumed at dusk, biologists would be time limited (<14 hours) for fish collection and stomach content retrieval.

For higher accuracy when examining diet composition, we recommend sampling during periods of cooler water (~17º C), as this will provide biologists with a longer amount of time to accurately identify digested prey. We also recommend sampling at dusk or early evening hours provide the highest likelihood of identifying stomach contents during summer months (25º C). Our results suggest that evening to early morning sampling during summer months would likely overestimate diel consumption rates when not considering mid-day or afternoon stomach contents. Conversely, sampling late morning to evening

would likely result in an underestimation of diel consumption rates. Researchers should consider whether sampling time is overestimating or underestimating results consumption rates.

Conclusion

Our work examining the effect of temperature on juvenile Flathead Catfish consumption, gastric evacuation, and intestinal passage of a single meal is a novel study of an apex predator. We found that warmer temperatures increase consumption and gastric evacuation rates of juvenile Flathead Catfish, while intestinal passage of a meal remained similar among our three treatment temperatures (17º C, 22º C, 25º C). Our results begin to shed light on effects of temperature on behavioral and physiological processes important for an individual's survival. Understanding how the environment influences physiological processes at the individual level provides biologists with knowledge at the population level. Knowledge of fish gastric evacuation rates are a necessary component for both field and laboratory studies when trying to understand feeding rates, modeling energy budgets, and understanding trophic dynamics of aquatic ecosystems. Ultimately, our work lays the foundation for future studies to examine the effect of temperature on bioenergetics of juvenile Flathead Catfish.

LITERATURE CITED

Andersen, N. G. 1999. The effects of predator size, temperature, and prey characteristics on gastric evacuation in Whiting. Journal of Fish Biology 54(2):287–301.

Andersen, N. G. 2012. Influences of potential predictor variables on gastric evacuation in Atlantic Cod *Gadus morhua* feeding on fish prey: parameterization of a generic model. Journal of Fish Biology 80(3):595–612.

Başçınar, N., N. S. Başçınar, K. Seyhan, and U. Khan. 2016. The effect of temperature on the rate of gastric evacuation in Brook Trout *Salvelinus fontinalis* fed on commercial pellets: group-feeding. Pakistan Journal of Zoology 48(6):1899-1904.

Bernreuther, M., A. Temming, and J. P. Herrmann. 2009. Effect of temperature on the gastric evacuation in Sprat *Sprattus sprattus*. Journal of Fish Biology 75(7):1525–1541.

Brett, J. R. and T. D. D. Groves. 1979. Physiological energetics. Fish Physiology 8(6):280-352.

Bourret, S. L., R. W. Tingley, Y. Kanno, and J. C. Vokoun. 2008. Maximum daily consumption and specific daily metabolic demand of juvenile Flathead Catfish *Pylodictis olivaris*. Journal of Freshwater Ecology 23(3):413–419.

Bromley, P. J. 1987. The effects of food type, meal size and body weight on digestion and gastric evacuation in Turbot *Scophthahnus maximits*. Journal of Fish Biology 30:501-512.

Bromley, P. J. 1994. The role of gastric evacuation experiments in quantifying the feeding rates of predatory fish. Reviews in Fish Biology and Fisheries 4(1):36–66.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85(7):1771–1789.

Brown, J. J., J. Perillo, T. J. Kwak, and R. J. Horwitz. 2005. Implications of *Pylodictis olivaris* (Flathead Catfish) introduction into the Delaware and Susquehanna drainages. Northeastern Naturalist 12(4):473–484.

Buckel, J. A., and D. O. Conover. 1996. Gastric evacuation rates of piscivorous young-of-the-year Bluefish. Transactions of the American Fisheries Society 125:591-599.

Buckmeier, D. L., E. R. Irwin, R. K. Betsill, and J. A. Prentice. 2002. Validity of otoliths and pectoral spines for estimating ages of Channel Catfish. Transactions of the American Fisheries Society 22:934-942.

Caissie, D. 2006. The thermal regime of rivers: a review. Freshwater Biology 51(8):1389–1406.

Chipps, S. R., D. F. Clapp, and D. H. Wahl. 2000. Variation in routine metabolism of juvenile Muskellunge: evidence for seasonal metabolic compensation in fishes. Journal of Fish Biology 56(2):311–318.

Craig, S., L. Helfrich, D. D. Kuhn, and M. H. Schwarz. 2017. Understanding fish nutrition, feeds, and feeding. Virginia Cooperative Extension.

Deslauriers, D., S. R. Chipps, J. E. Breck, J. A. Rice, and C. P. Madenjian. 2017. Fish bioenergetics 4.0: an R-based modeling application. Fisheries 42(11):586–596.

Eddy, B., and R. D. Handy. 2012. Ecological and environmental physiology of fishes. Oxford University Press, Oxford.

Elliott, J. M. 1972. Rates of gastric evacuation in Brown Trout, *Salmo trutta*. Freshwater Biology 2(1):1–18.

Elliott, J. M., and Persson, L. 1978. The estimation of daily rates of food consumption for fish. Journal of Animal Ecology 47:977–991.

Fuller, P. L., and G. E. Whelan. 2018. The Flathead Catfish invasion of the Great Lakes. Journal of Great Lakes Research 44(5):1081–1092.

Gammon, J. R. 1973. The effect of thermal effluents on the populations of fish and macroinvertebrates in the Wabash River. Purdue University Water Research Center, Technical Report 32, Lafayette, Indiana.

Glenn, C. L., and F. J. Ward. 1968. "Wet" weight as a method for measuring stomach contents of Walleyes, *Stizostedion vitreum vitreum*. Journal of the Fisheries Board of Canada 25(7):1505-1507.

Grove, D. J., L. G. Loizides, and J. Nott. 1978. Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. Journal of Fish Biology 12(5):507-516.

He, E., and W. A. Wurtsbaugh. 1993. An empirical model of gastric evacuation rates for fish and an analysis of digestion in piscivorous Brown Trout. Transactions of the American Fisheries Society 122(5):717-730.

Hanson, P. C., T. B. Johnson, D. E. Schindler and J. F. Kitchell. 1997. Fish bioenergetics 3.0 Madison: University of Wisconsin Sea Grant Institute. Report WISCU-T-97–001.

Helmuth, B., J. G. Kingsolver, and E. Carrington. 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? Annual Review of Physiology 67:177-201.

Horodysky, A. Z., S. J. Cooke, and R. W. Brill. 2015. Physiology in the service of fisheries science: why thinking mechanistically matters. Reviews in Fish Biology and Fisheries 25(3):425–447.

Hrabik, R.A., S.C. Schainost, R.H. Stasiak, and E.J. Peters. 2015. The fishes of Nebraska. Mennonite Press, Newton, Kansas.

Jobling, M. 1980. Gastric evacuation in Plaice, *Pleuronectes platessa*: effects of temperature and fish size. Journal of Fish Biology 17:547-551.

Kapoor, B. G., H. Smit, and I. A. Verighina. 1975. Digestion rate in teleosts. Pages 180-188 *In* F. S. Russell and M. Yonge, editors. Advances in Marine Biology. Academic Press, New York.

Langton, R. W. 1977. A review of methods used for estimating gut evacuation rates and calculating daily ration for fish. Woods Hole Laboratory Reference 77-07, 1-26. Available: https://www.nefsc.noaa.gov/publications/series/whlrd/whlrd7707.pdf

Legler, N. D., T. B. Johnson, D. D. Heath, and S. A. Ludsin. 2010. Water temperature and prey size effects on the rate of digestion of larval and early juvenile fish. Transactions of the American Fisheries Society 139(3):868–875.

Lenth, R.V. and M. Hervé. 2015. Package 'lsmeans'. Available: cran.r-project.org/web/packages/lsmeans/lsmeans.pdf

Ling, K. M., and M. A. Ghaffar. 2014. Estimation of gastric emptying time (GET) in Clownfish *Amphiprion ocellaris* using x-radiography technique. *In* AIP Conference Proceedings 1614:624-628. Available: https://aip.scitation.org/doi/abs/10.1063/1.4895275

Mahoney, E. T. 1982. The Missouri River Channel Catfish. Nebraska Game and Parks Commission white pages, conference presentations, and manuscripts 32.

Magnuson, J. J. 1969. Digestion and food consumption by Skipjack Tuna *Katsuwonus pelamis.* Transactions of the American Fisheries Society 98(3):379–392.

Minckley, W. L., and J. E. Deacon. 1959. Biology of the Flathead Catfish in Kansas. Transactions of the American Fisheries Society 88(4):344-355.

Minton, J. W., and R. B. McLean. 1982. Measurements of growth and consumption of Sauger *Stizostedion canadense*: implication for fish energetics studies. Canadian Journal of Fisheries and Aquatic Sciences 39(10):1396–1403.

Munger, C. R., G. R. Wilde, and B. J. Follis. 1994. Flathead Catfish age and size at maturation in Texas. North American Journal of Fisheries Management 14(2):403-408.

Nakagawa, H. 2018. Temperature-dependent gastric evacuation rate of the Japanese Delicate Loach *Niwaella delicate.* Ichthyological Research 65(1):172–174.

Ndome, C. B., A. O. Ekwu, and A. A. Ateb. 2011. Effect of feeding frequency on feed consumption, growth and feed conversion of *Clarias gariepinus* male and *Heterobranchus longifilis* female hybrids in glass aquaria. American-Eurasian Journal of Scinetific Research 6:1:6-12.

Ogle, D. H. 2016. Introductory fisheries analyses with R. Chapman and Hall/CRC Press, Boca Raton, FL.

Perrson, L. 1979. The effects of temperature and different food organisms on the rate of gastric evacuation in perch (*Perca fluviatilis*). Freshwater Biology 9:99-104.

Pine, W. E., III. T. J. Kwak, D. S. Waters, and J. A. Rice. 2005. Diet selectivity of introduced Flathead Catfish in coastal rivers. Transactions of the American Fisheries Society 134(4):901–909.

Quinn, S. P. 1988. Flathead Catfish abundance and growth in the Flint River, Georgia.Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 42(1988):141-148.

Riche, M., D. I. Haley, M. Oetker, S. Garbrecht, and D. L. Garling. 2004. Effect of feeding frequency on gastric evacuation and the return of appetite in Tilapia *Oreochromis niloticus*. Aquaculture 234(1-4): 657-673.

Rijnsdorp, A. D. 1990. The mechanism of energy allocation over reproduction and somatic growth in female North Sea plaice, *Pleuronectes platessa*. Netherlands Journal of Sea Research 25(1-2): 279-289.

Roell, M. J., and D. J. Orth. 1993. Trophic basis of production of stream-dwelling Smallmouth Bass, Rock Bass, and Flathead Catfish in relation to invertebrate bait harvest. Transactions of the American Fisheries Society 122(1):46–62.

Rønnestad, I., A. S. Gomes, K. Murashita, R. Angotzi, E. Jönsson, and H. Volkoff. 2017. Appetite-controlling endocrine systems in teleosts. Frontiers in Endocrinology 8(73).

Seyhan, K., D. J. Grove and J. King. 1998. Feeding behaviour of Whiting *Merlangius merlangus* in captivity. Fisheries Research 34(1):39–45.

Seyhan, K., and D. J. Grove. 2003. A new approach in modelling gastric emptying in fish. Turkish Journal of Veterinary and Animal Sciences 27(5):1043–1047.

Sims, D. W., S. J. Davies and Q. Bone. 1996. Gastric emptying rate and return of appetite in Lesser Spotted Dogfish *Scyliorhinus canicula* (Chondrichthyes: Elasmobranchii). Journal of the Marine Biological Association of the United Kingdom 76(2):479-491.

Smith, R. R. 1980. Nutritional bioenergetics in fish. *In* K. W. Chow, editor. Fish Feed Technology. FAO (Food and Agriculture Organization of the United Nations), ADCP/REP/80/11, Rome. Available: http://www.fao.org/3/x5738e/x5738e00.htm#Contents.

Sneed, K. E. 1951. A method for calculating the growth of Channel Catfish, *Ictalurus Lacustris Punctatus*. Transactions of the American Fisheries Society 80(1):174–183.

Sweka, J. A., M. K. Cox, and K. J. Hartman. 2004. Gastric evacuation rates of Brook Trout. Transactions of the American Fisheries Society 133(1):204–210.

Swenson, W. A., and L. L. Smith Jr. 1973. Gastric digestion, food consumption, feeding periodicity, and food conversion efficiency in Walleye *Stizostedion vitreum vitreum*. Journal of the Fisheries Research Board of Canada 30(9):1327–1336.

Talbot, C. 1985. Laboratory methods in fish feeding and nutritional studies. Pages 125-154 *in* P. Tytler and P. Calow, editors. Fish Energetics: new perspectives. Croom Helm, London.

Tetzlaff, J. C., H. J. Flowers and W. E. Pine, III. 2010. Consumption and growth patterns of Flathead Catfish derived from a bioenergetics model. Open Fish Science Journal 3(1):101–109.

Turner, D. R. 2017. Trophic dynamics of Flathead Catfish in the Missouri River bordering Nebraska. Master's thesis. University of Nebraska, Lincoln.

Tyler, A. V. 1970. Rates of gastric emptying in young cod. Journal of the Fisheries Research Board of Canada 27(7):1177–1189.

USGS (United States Geological Survey). 2019. USGS water data for the nation [online database]. United States Geological Survey, Reston, Virginia. Available: https://waterdata.usgs.gov/usa/nwis/uv?site_no=06807000

Waters, D. S., T. J. Kwak, J. B. Arnott, and W. E. Pine, III. 2004. Evaluation of stomach tubes and gastric lavage for sampling diets from Blue Catfish and Flathead Catfish. North American Journal of Fisheries Management 24(1):258–261.

Whitledge, G. W., P. G. Bajer, and R. S. Hayward. 2010. Laboratory evaluation of two bioenergetics models for Brown Trout. Transactions of the American Fisheries Society, *139*(4): 929–936.

Windell, J. T., and D. O. Norris. 1969. Gastric digestion and evacuation in Rainbow Trout. The Progressive Fish-Culturist 31(1):20–26.

Wootton, R. J., and C. Smith. 2015.Pages 109-110. *In* Reproductive biology of teleost fishes. Wiley-Blackwell, Oxford, UK.

Zar, J. H. 2010. Biostatistical analysis, 5th edition. Pearson Prentice-Hall. Upper Saddle River, New Jersey.

Table 2-1. Summary information of the mean total length (mm) and weight (g) of Flathead Catfish, mean Goldfish weight (g), and mean ration size (meal size as % body weight of Flathead Catfish), count of male, female, and unknown Flathead Catfish among all treatments combined (n = 210 Flathead Catfish).

N	Mean ± (SD) Total Length, mm	Mean ± (SD) Weight, g	Mean ± (SD) Goldfish Weight, g	Mean ± (SD) Ration Size, %	Male	Female	Unknown
126	300 (50)	255 (125)	3.01 (1.08)	1.5 (1.01)	55	52	19

(SD) = Standard deviation

Table 2-2. Results of an ANOVA ($\alpha = 0.05$) of sex, age, and temperature on gonadosomatic index values of Flathead Catfish.

	Df	Deviance	Residual Df	Residual Deviance	Pr(>Chi)
Null			84	5.51	
Sex	1	1.26	83	4.25	< **0.001**
Age	4	1.45	79	2.80	< **0.001**
Treatment	2	0.12	77	2.68	0.178

Dependent variable = Gonadosomatic index
Df = degrees of freedom

Table 2-3. Summary information of water quality parameters. Mean and standard deviation (SD) of ammonia, nitrite, nitrate and pH levels for each system throughout the duration of the experiment. Differences among means were tested with ANOVA ($\alpha = 0.05$).

System	N	Mean ± (SD) Ammonia	Mean ± (SD) Nitrite	Mean ± (SD) Nitrate	Mean ± (SD) pH
1	20	0.13 (0.15)	0.33 (1.12)	44.63 (37.80)	8.20 (0.09)
2	20	0.11 (0.12)	0.26 (0.61)	43.50 (34.07)	8.19 (0.05)
3	20	0.09 (0.15)	0.02 (0.06)	46.25 (37.02)	8.21 (0.09)

(SD) = Standard deviation

Table 2-4. Generalized linear model (family binomial) selection, using Akaike information criterion (AICc), for consumption of Goldfish by juvenile Flathead Catfish. We examined temperature (º C), prey weight (wet weight of Goldfish), and experimental tanks as predictors of consumption.

Model Name	K	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log-likelihood
Prey Weight + Temperature	4	263.25	0.00	0.48	0.48	-127.53
Temperature	3	264.10	0.85	0.31	0.79	-128.99
Prey Weight x Temperature	6	265.68	2.43	0.14	0.93	-126.63
Temperature + Prey Weight + Experimental Tank	10	268.07	4.82	0.04	0.97	-123.48
Experimental Tank	8	269.13	5.88	0.03	1.00	-126.20
Prey Weight	2	282.16	18.91	0.00	1.00	-139.05
Null	1	282.84	19.60	0.00	1.00	-140.41

Dependent variable = Consumption.
K = number of paramters

Table 2-5. Examination of two supporting models on consumption of Goldfish by juvenile Flathead Catfish. Differences among means were tested with ANOVA ($\alpha = 0.05$).

Model	Df	Deviance	Residual Df	Residual Deviance	Pr(>Chi)
1			206	257.985	
2	1	2.934	205	255.056	0.087

Model 1: Consumption ~ Temperature
Model 2: Consumption ~ Temperature + Prey Weight
Df = degrees of freedom

Table 2-6. Results of a post-hoc analysis using least square means for multiple comparison (Tukey-adjusted comparison) to examine differences in consumption between treatments (17º C, 22º C, 25º C). Mean consumption events between treatments are different based on $\alpha = 0.05$.

	Estimate	**SE**	**Df**	**z-ratio**	**p-value**
17º C - 22º C	-0.67	0.34	Inf	-1.94	0.127
17º C - 25º C	-1.73	0.39	Inf	-4.50	**< 0.001**
22º C - 25º C	-1.06	0.40	Inf	-2.66	**0.021**

SE = standard error
Df = degrees of freedom

Table 2-7. Generalized linear model (family binomial) selection, using Akaike information criterion (AICc), for gastric evacuation rates of juvenile Flathead Catfish. We examined digestion time (hrs), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), experimental tank, sex, and age as predictors of the proportion of the meal remaining in the stomach (wet weight of stomach content divided by initial wet weight of Goldfish) of juvenile Flathead Catfish.

	K	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log-likelihood
Digestion Time + Temperature + Ration Size	5	151.45	0.00	0.90	0.90	-70.47
Digestion Time * Ration Size	11	156.08	4.64	0.09	0.99	-65.88
Global	10	162.47	11.02	0.00	1.00	-70.28
Digestion Time * Temperature	12	163.13	11.68	0.00	1.00	-68.18
Digestion Time * Temperature * Ration Size	17	168.59	17.14	0.00	1.00	-64.46
Digestion Time	2	168.70	17.26	0.00	1.00	-82.30
Temperature	3	372.49	221.04	0.00	1.00	-183.15
Ration Size	2	373.38	221.93	0.00	1.00	-184.64
Null	1	375.10	223.65	0.00	1.00	-186.53

Dependent variable: Proportion of meal remaining in the stomach
K = number of model parameters

Table 2-8. Results of an ANOVA (α= 0.05) of digestion time, temperature, and ration size on the proportion of the meal remaining (wet weight of stomach contents divided by initial wet weight of Goldfish fed to Flathead Catfish) in the stomachs of juvenile Flathead Catfish.

	Df	Deviance	Residual Df	Residual Deviance	Pr(>Chi)
Null			125	252.51	
Digestion Time	1	195.93	124	56.59	**< 0.001**
Temperature	2	11.77	122	44.82	**0.003**
Ration Size	1	8.25	121	36.56	**0.004**

Dependent variable = Proportion of meal remaining in the stomach
Df = degrees of freedom

Table 2-9. Results of a post-hoc analysis using least square means for multiple comparison (Tukey-adjusted comparison) to examine differences in gastric evacuation rates between treatments (17º C, 22º C, 25º C) of juvenile Flathead Catfish. Mean gastric evacuation rates between treatments were different based on $\alpha = 0.05$.

	Estimate	**SE**	**Df**	**z-ratio**	**p-value**
17º C -22º C	0.90	0.42	Inf	2.17	0.076
17º C -25º C	1.30	0.39	Inf	3.35	**0.002**
22º C -25º C	0.40	0.35	Inf	1.14	0.490

SE = standard error
Df = degrees of freedom

Table 2-10. Estimated time (hours) to reach a given percentage of stomach evacuation for juvenile Flathead Catfish. Gastric emptying time (time at which the stomach is empty) was based on a 90% evacuation of the meal from the stomach and is shown in bold.

Evacuated	17º C	22º C	25º C
0 %	0.00	0.00	0.00
25 %	6.84	3.34	1.77
50 %	11.10	7.60	6.05
75 %	15.38	11.88	10.32
90 %	**19.65**	**16.15**	**14.55**
95 %	22.50	19.05	17.50
100 %	32.00	29.00	27.00

Table 2-11. Generalized linear model (family binomial) selection, using Akaike information criterion (AICc), for intestinal passage rates of juvenile Flathead Catfish fed a single meal. We examined digestion time (hrs), digestion time^2 (Fit; a fitted parabola function to capture the increase and decrease of contents through time), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), experimental tank, sex, and age as predictors of the proportion of the meal remaining in the intestines (wet weight of intestinal content divided by initial wet weight of Goldfish) of juvenile Flathead Catfish.

	K	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log-likelihood
Digestion Time + Ration Size + Fit	4	211.38	0.00	0.32	0.32	-101.53
Ration Size + Fit	3	211.84	0.45	0.25	0.57	-102.82
Digestion Time + Ration Size + Temperature + Fit	6	211.86	0.48	0.25	0.82	-99.58
Digestion Time + Ration Size	3	214.57	3.19	0.06	0.89	-104.19
Digestion Time + Ration Size + Temperature	5	214.77	3.39	0.06	0.95	-102.14
Digestion Time + Fit	3	217.23	5.85	0.02	0.96	-105.52
Fit + Temperature	4	217.49	6.11	0.02	0.98	-104.58
Ration Size	2	218.63	7.24	0.01	0.99	-107.26
Digestion Time + Temperature	4	220.04	8.66	0.00	0.99	-105.85
Digestion Time	2	220.14	8.75	0.00	1.00	-108.02
Global	11	222.17	10.79	0.00	1.00	-98.93
Digestion Time * Ration Size	12	223.53	12.14	0.00	1.00	-98.38
Null	1	223.95	12.57	0.00	1.00	-110.96
Temperature	3	223.99	12.61	0.00	1.00	-108.90
Digestion Time * Temperature	13	225.96	14.57	0.00	1.00	-98.35
Digestion Time *Temperature * Ration Size	18	236.51	25.12	0.00	1.00	-97.06

Dependent variable = Proportion of meal remaining within the intestines
K = number of parameters

Table 2-12. Results of an ANOVA (α= 0.05) for three supporting models of the proportion of the meal remaining in the intestines of juvenile Flathead Catfish.

Model		Df	Deviance	Residual Df	Residual Deviance	Pr(>Chi)
1	Null			125	43.73	
	Digestion Time	1	4.76	124	38.96	**0.029**
	Fit	1	3.73	123	35.23	0.053
	Ration Size	1	6.96	122	28.27	**0.008**
2	Null			125	47.73	
	Fit	1	6.76	124	36.70	**0.009**
	Ration Size	1	6.76	123	30.21	**0.009**
3	Null			125	43.73	
	Digestion Time	1	4.76	124	38.96	**0.029**
	Fit	1	3.73	123	35.23	0.053
	Ration Size	1	6.96	122	28.27	**0.008**
	Temperature	2	4.47	120	23.80	0.107

Dependent variable = Proportion of meal remaining in the intestines
Df = degrees of freedom

Table 2-13. One-way ANOVA (α= 0.05) examining light status (on or off) effect on the count of juvenile Flathead Catfish that consumed a Goldfish for each one-hour time interval of a 24-hour day.

	Df	**Sum of Squares**	**Mean Sum of Square**	**F-value**	**Pr(>F)**
On_Off	1	160.2	160.2	6.619	**0.017**
Residuals	22	532.3	24.2		

Dependent variable = Count of Flathead Catfish to consume a Goldfish
Df = degrees of freedom

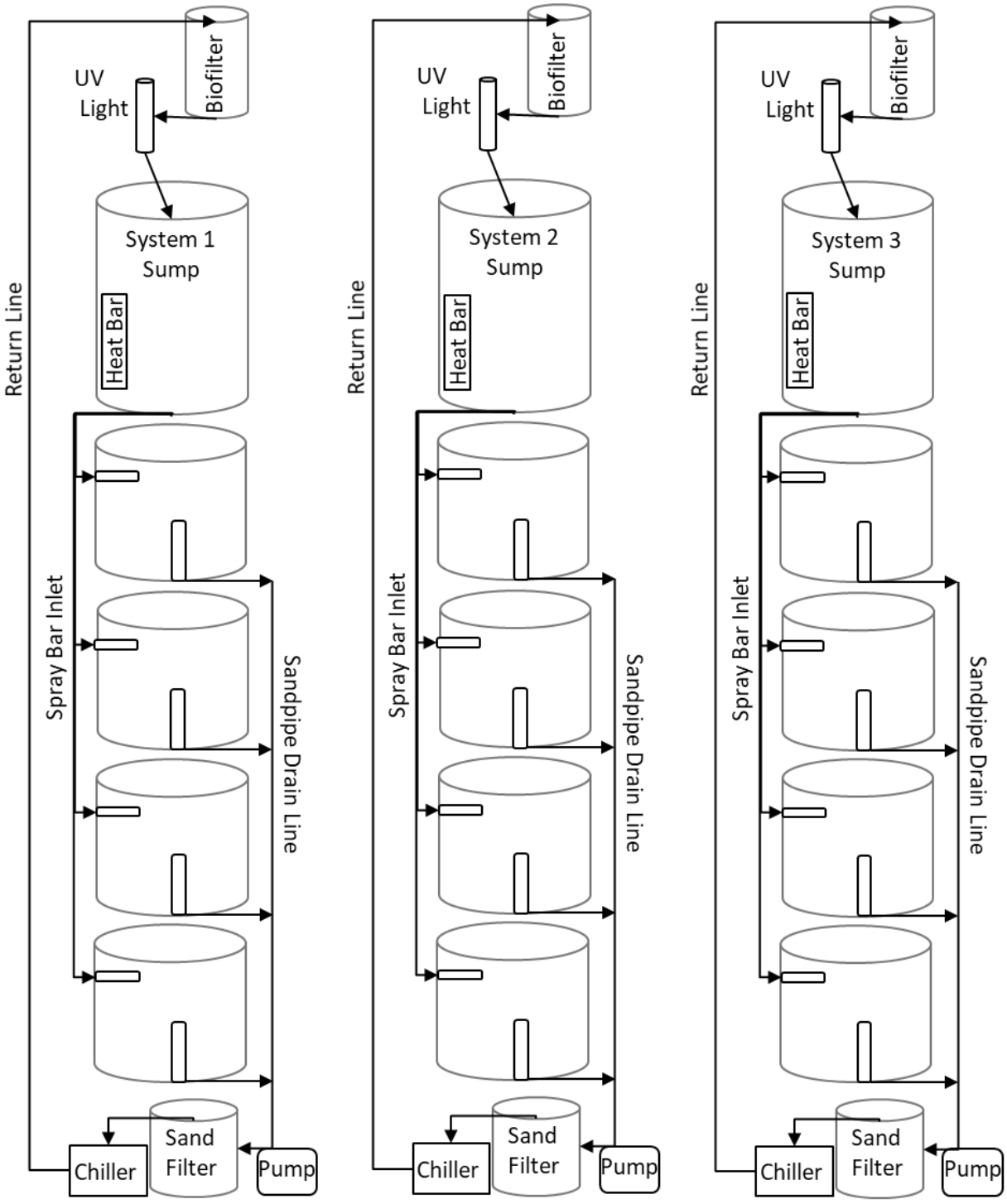


Figure 2-1. Conceptual diagram of the recirculating aquatic system within the University of Nebraska - Lincoln Fish Conservation, Behavior, and Physiology Laboratory.

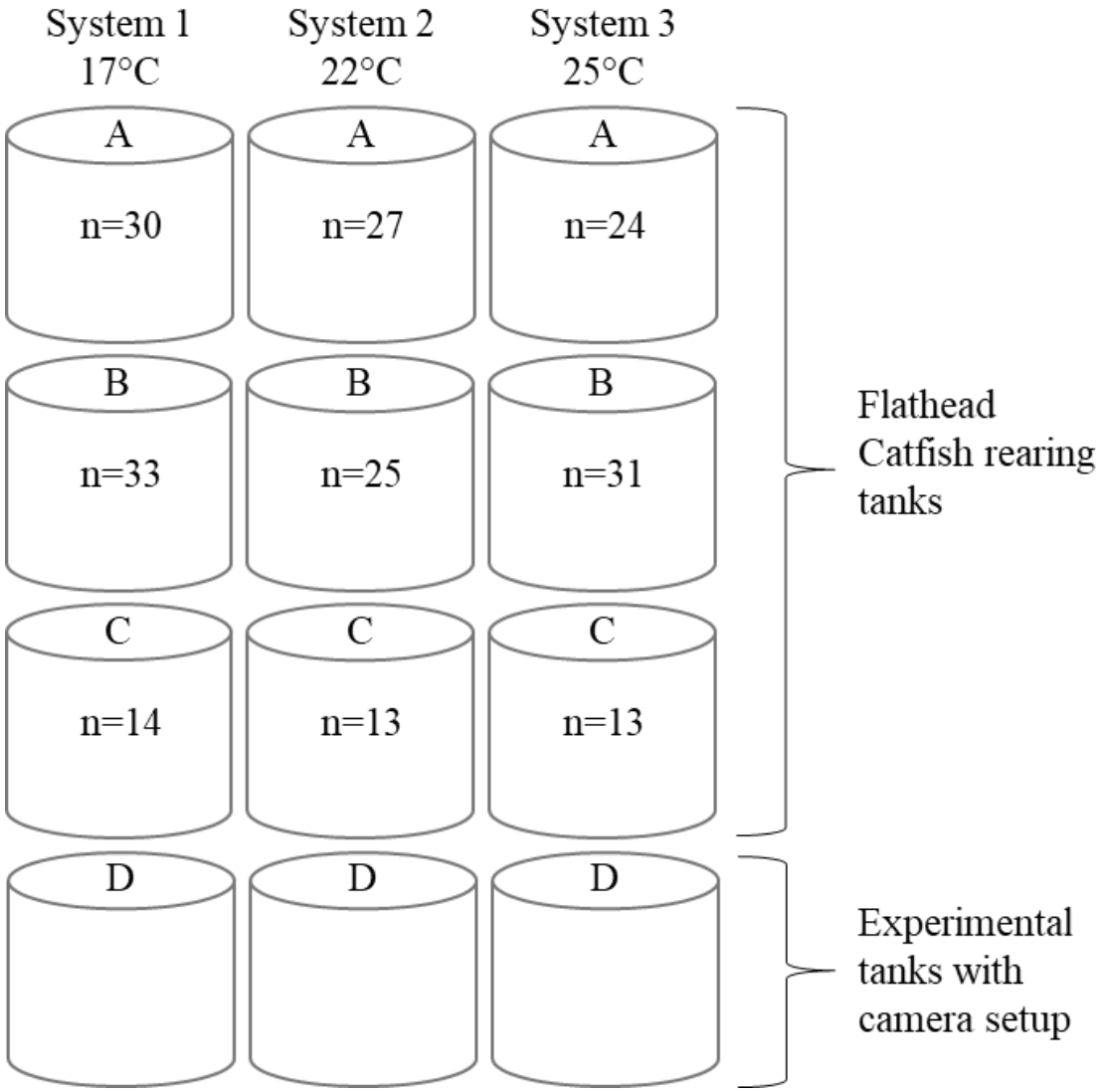


Figure 2-2. Conceptual diagram of random distribution of Flathead Catfish (n = 210) among three treatment (17º C, 22º C, and 25º C) groups. Tank D of each system was left empty for experimental aquaria setup.

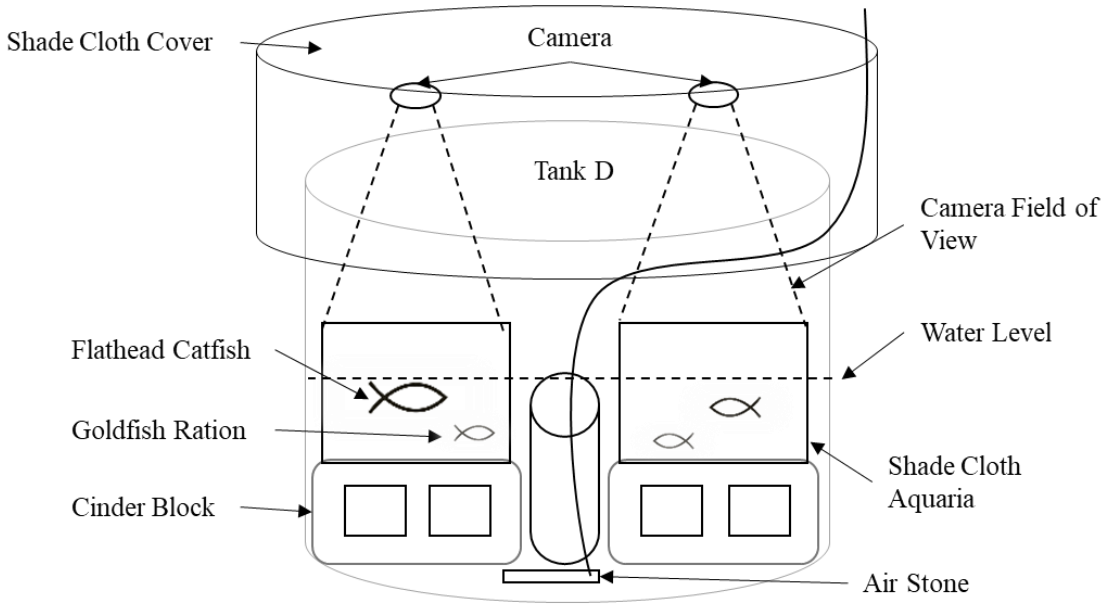


Figure 2-3. Conceptual diagram of the experimental aquaria setup in tank D of a recirculating aquatic system. Water flowed freely between the experimental aquaria and the surrounding tank. A Flathead Catfish and a Goldfish of known wet weight were placed within an experimental aquarium. Cameras above each experimental aquaria were used to monitor consumption.

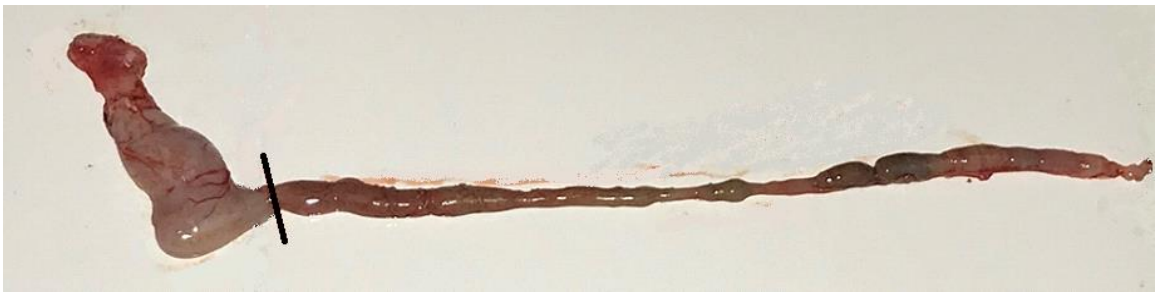


Figure 2-4. A photo of the digestive tract of a Flathead Catfish. Food passes from the esophagus into the stomach before passing into intestines and expelling from the anus. The solid line indicates where the stomach empties into the intestines.

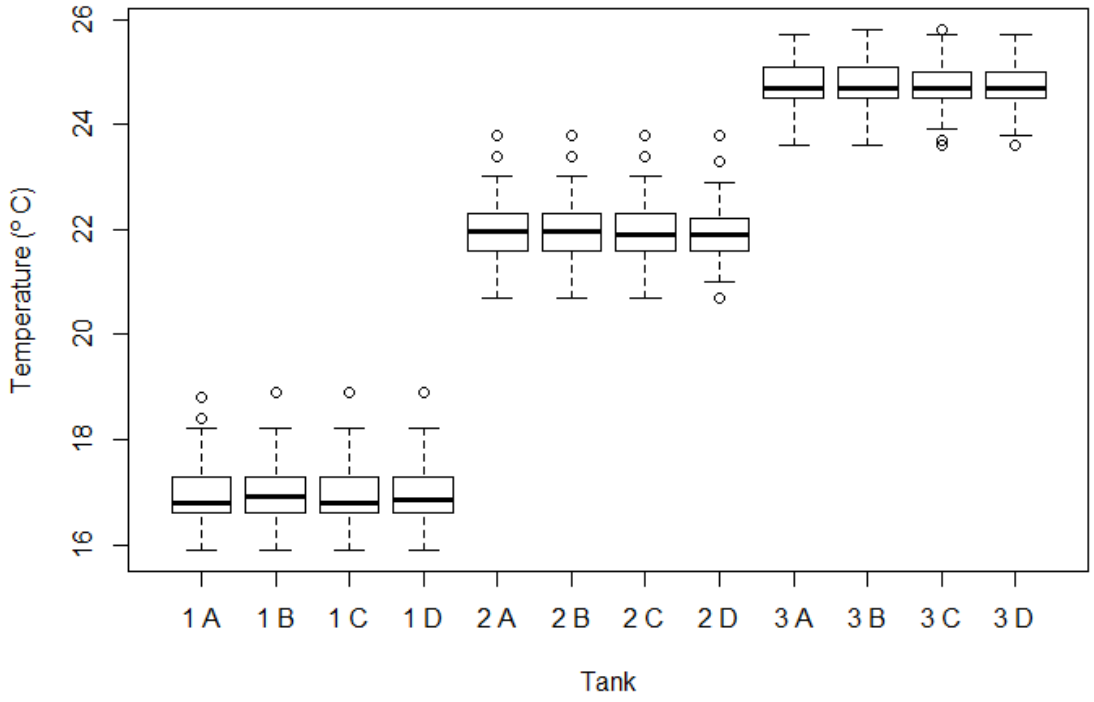


Figure 2-5. Box and whisker plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of temperature among tanks of each system. Temperature was not different among tanks within each system; system 1 ($F_{3,532} = 0.026$, P = 0.994), system 2 ($F_{3,532} = 0.059$, P = 0.981), and system 3 ($F_{3,420} = 0.481$, P = 0.696). Differences were tested with ANOVA ($\alpha = 0.05$).

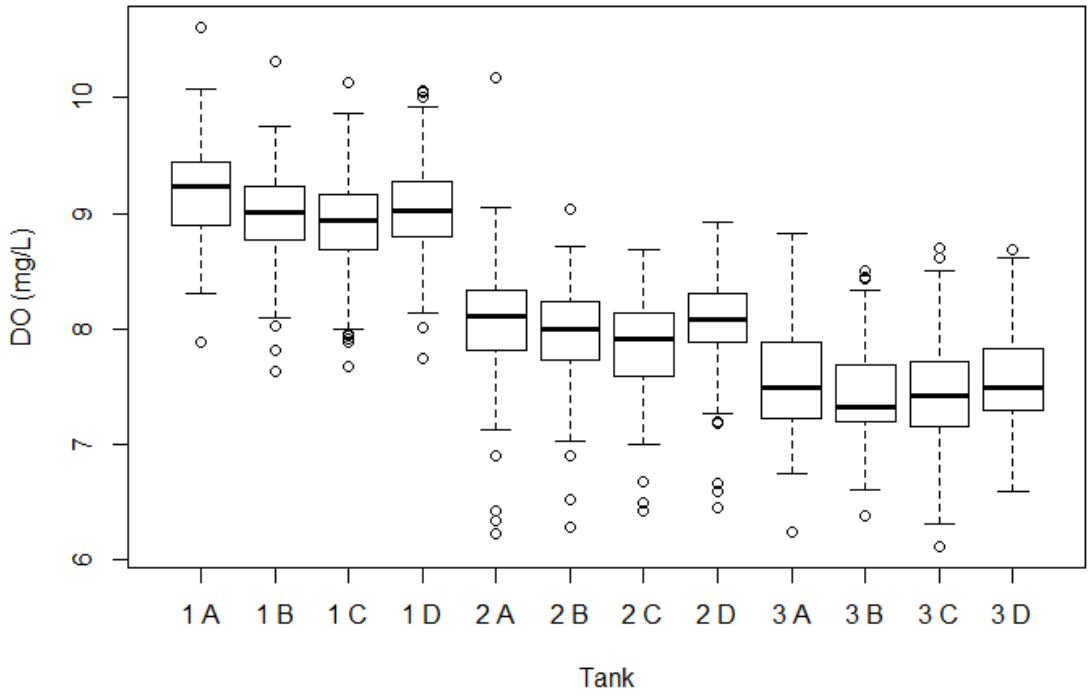


Figure 2-6. Box and whisker plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of dissolved oxygen (mg/L) among tanks of each system. Dissolved oxygen was different among tanks within each system; system 1 ($F_{3,532} = 11.61$, $P < 0.001$), system 2 ($F_{3,532} = 6.631$, $P < 0.001$), and system 3 ($F_{3,420} = 2.809$, $P = 0.039$). Differences were tested with ANOVA ($\alpha = 0.05$).

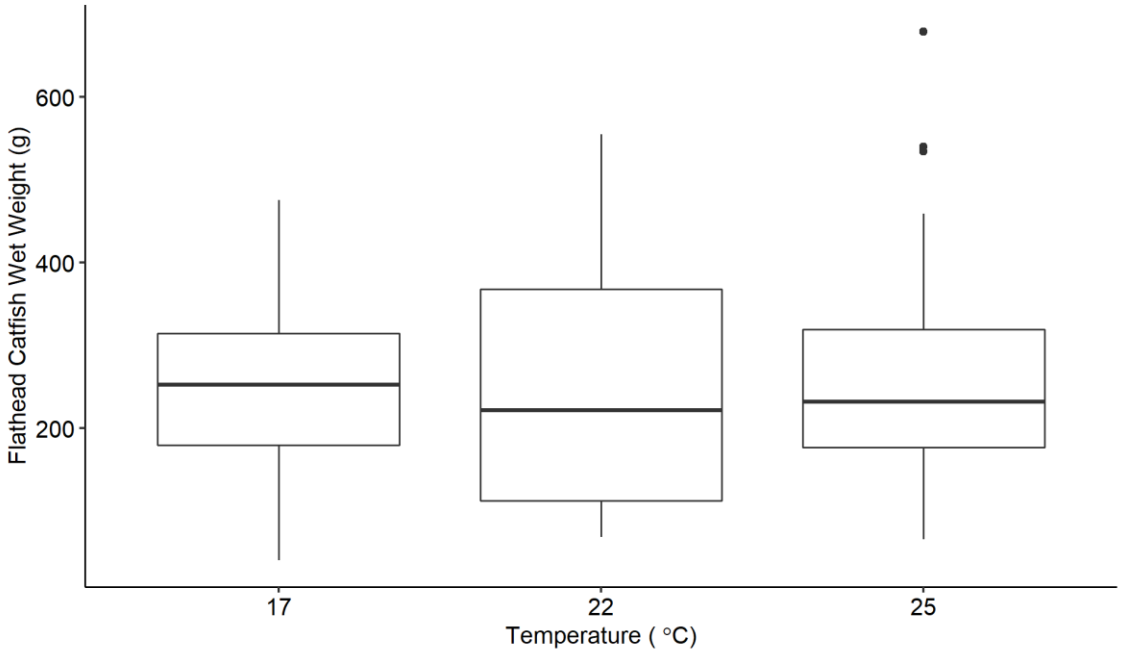


Figure 2-7. Box plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of Flathead Catfish weights (g) for each treatment. Flathead Catfish weight was not different ($F_{2,123} = 0.374$, $P = 0.689$) among treatments. Differences were tested with ANOVA ($\alpha = 0.05$).

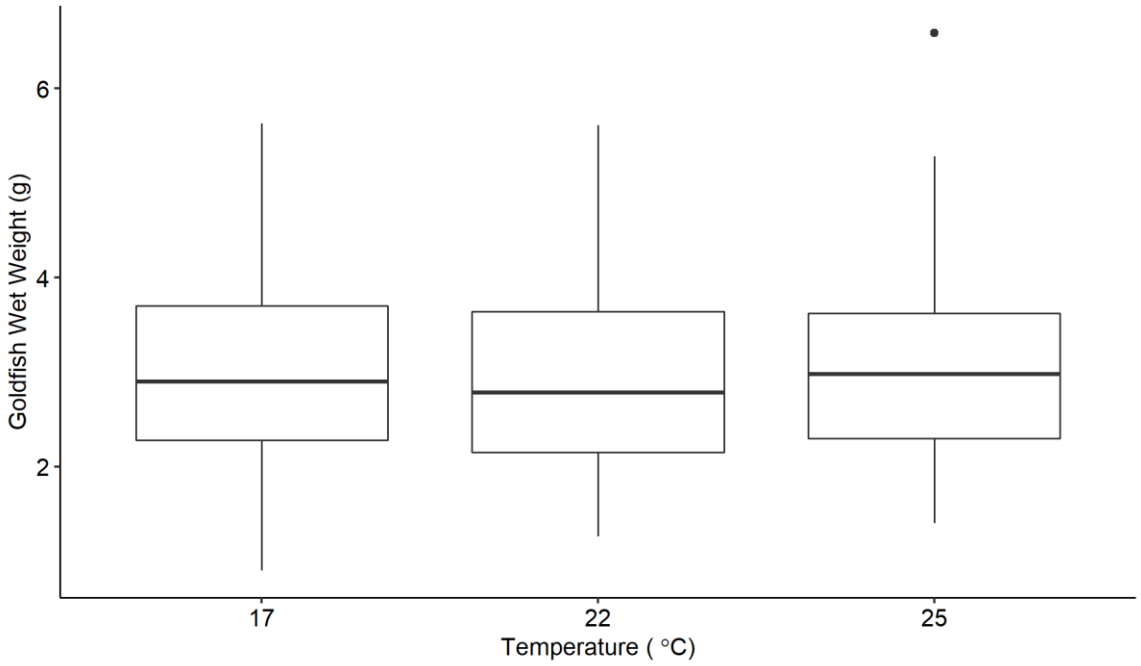


Figure 2-8. Box plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of initial Goldfish weights (g) that were consumed by Flathead Catfish for each treatment. Goldfish weights were not different ($F_{2,123} = 0.283$, $P = 0.754$) among treatments. Differences were tested with ANOVA ($\alpha = 0.05$).

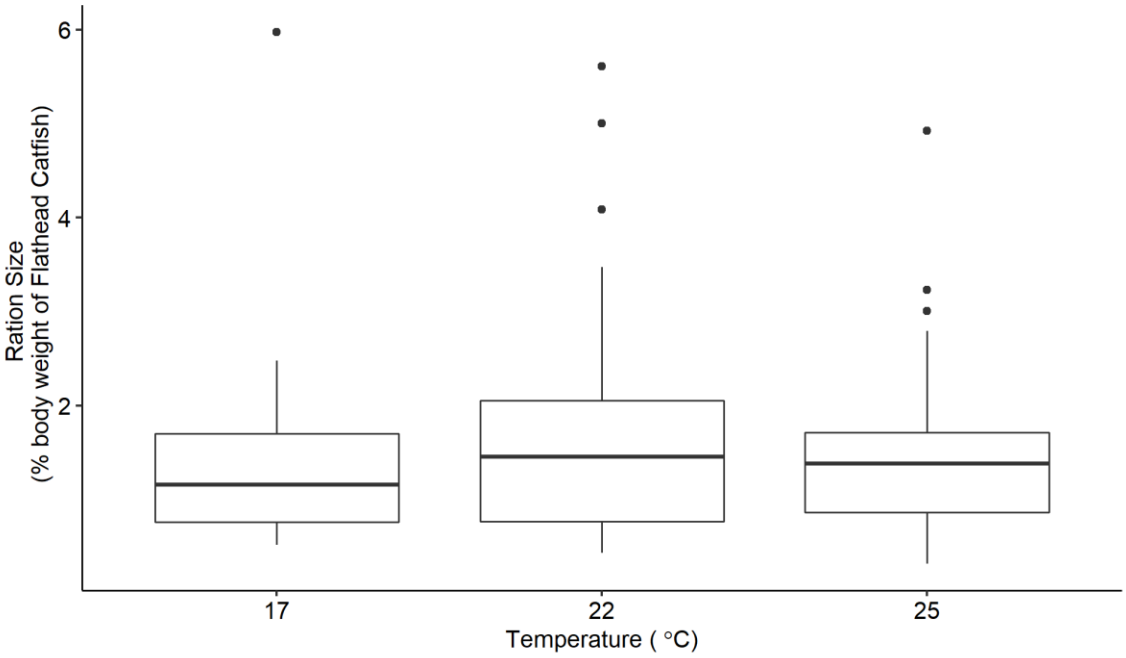


Figure 2-9. Box plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) of ration sizes that were consumed by Flathead Catfish for each treatment. Goldfish weights were not different ($F_{2,123} = 0.945$, $P = 0.392$) among treatments. Differences were tested with ANOVA ($\alpha = 0.05$).

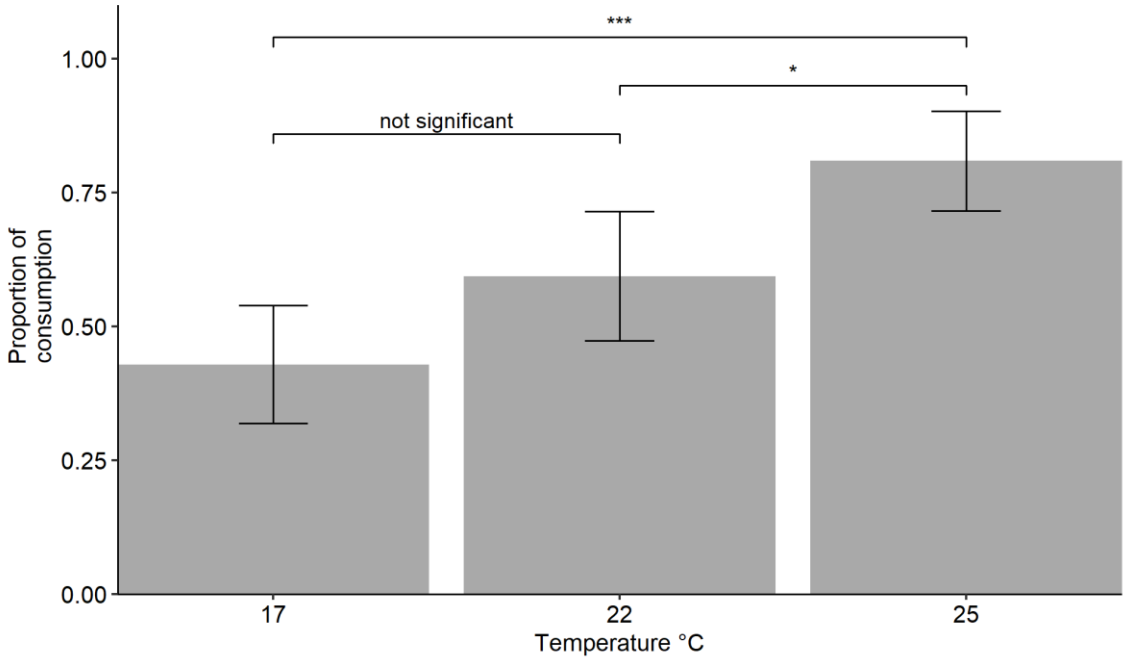


Figure 2-10. Bar graph (with mean and 95% CI) representing the proportion of consumption (number of Flathead Catfish that consumed a Goldfish divided by the total number of trials) that occurred within each treatment temperature (17º C, 22º C, and 25º C). Consumption of Goldfish increased as temperatures increased. A proportion of 0.429 (± 0.111) of Flathead Catfish ate at 17º C, 0.594 (± 0.120) of Flathead Catfish ate at 22º C, and .809 (± 0.093) of Flathead Catfish ate at 25º C. There was a significant difference between 17º C and 25º C ($P < 0.001$) and 22º C and 25º C ($P = 0.021$) treatments, but not 17º C and 22º C ($P = 0.127$).

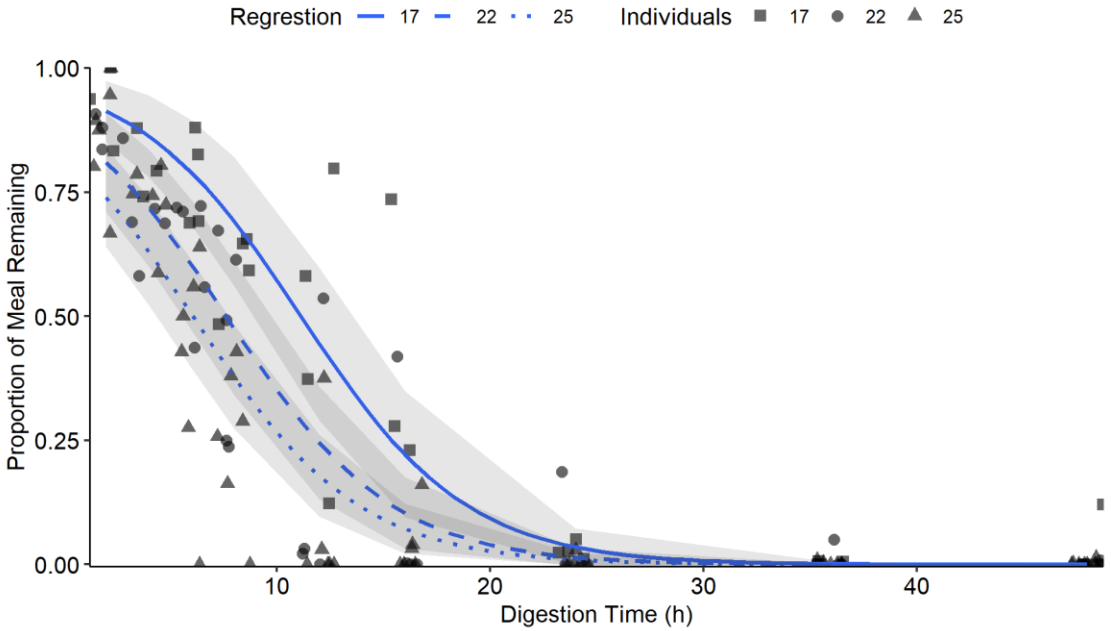


Figure 2-11. Binomial logistic regression for each treatment (17º C, solid line; 22º C, dashed line; and 25º C, dotted line) on the proportion of the meal remaining in stomach of juvenile Flathead Catfish (n =126); based on nine digestion times (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h) and a mean ration size of 0.015. Regression overlays the proportion of the meal remaining in the stomach of dissected individuals (17º C, squares, n = 33; 22º C, circles, n = 38; and 25º C, triangles, n = 55). Gastric evacuation rates were different between 17º C and 25º C (P = 0.002), but not 17º C and 22º C (P = 0.076) and 22º C and 25º C (P = 0.490).

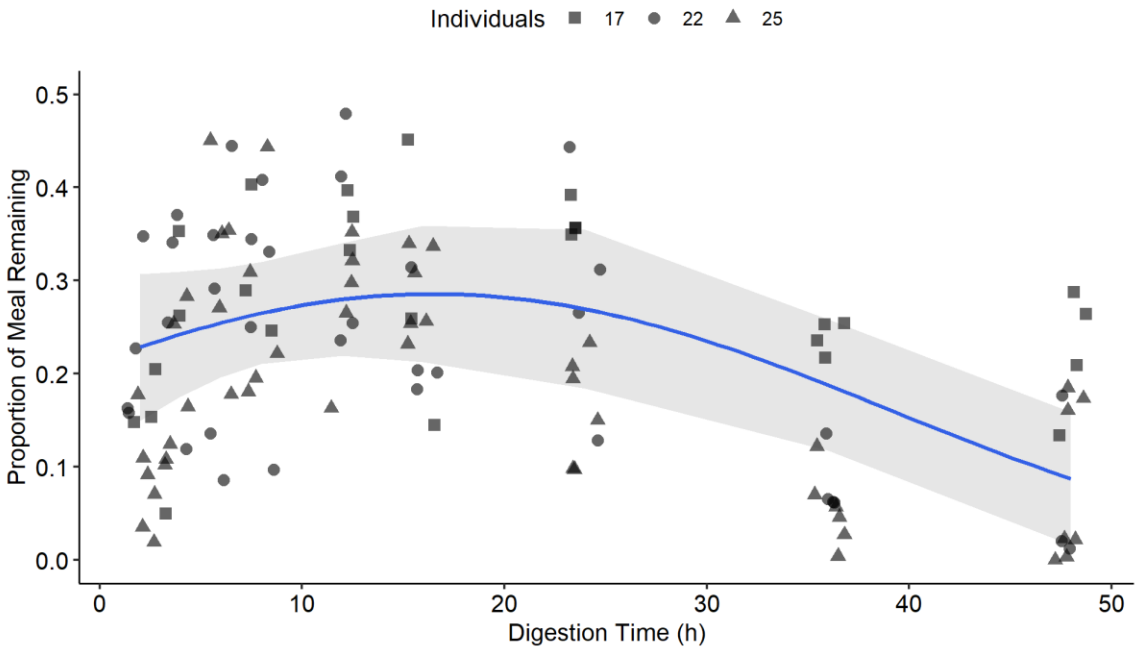


Figure 2-12. Binomial logistic regression on the proportion of the meal remaining in intestines of juvenile Flathead Catfish (n =126); based on nine digestion times (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h) and a mean ration size of 0.015. Regression overlays the proportion of the meal remaining in the intestines of dissected individuals (17º C, squares, n = 33; 22º C, circles, n = 38; and 25º C, triangles, n = 55).

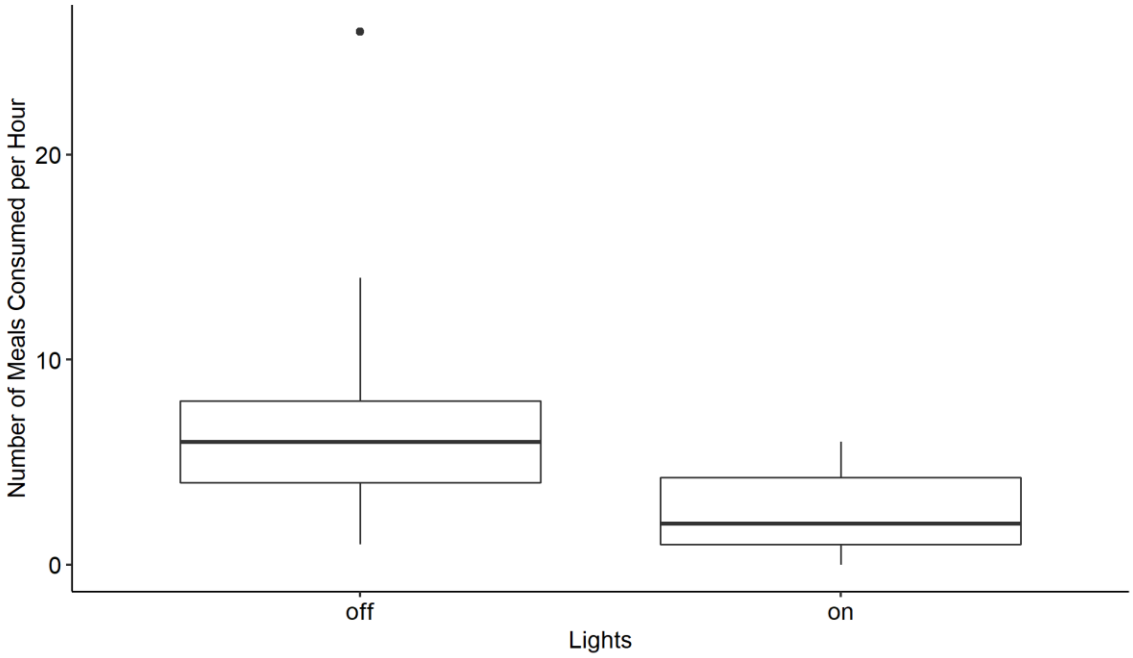


Figure 2-13. Box plot (whiskers = smallest or largest observations less than or equal to lower or upper hinge + 1.5 * IQR; box = 25 and 75% percentiles and median) on the number of meals consumed per hour interval by juvenile Flathead Catfish during lights off and lights on in the laboratory.

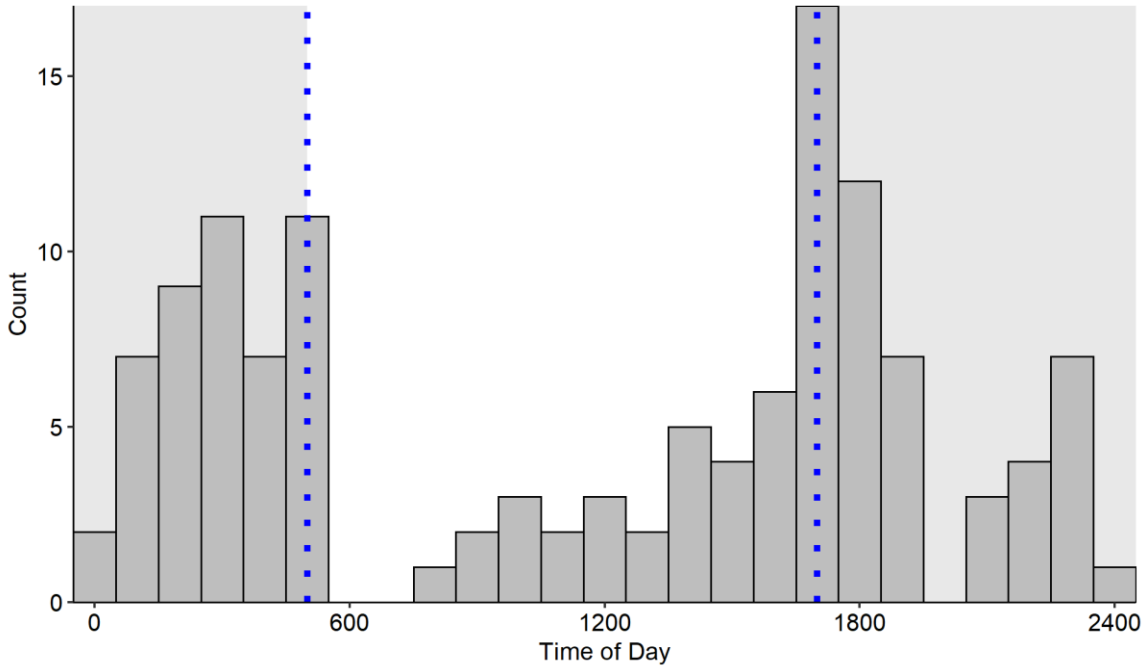


Figure 2-14. Bar graph showing the cumulative count of juvenile Flathead Catfish that consumed a Goldfish for each hour of the day. Dashed blue lines indicate lights on (0545) and lights off (1745) in the laboratory. Of the 126 Flathead Catfish to consume a Goldfish, 28 individuals ate during lights on and 98 individuals ate during lights off.

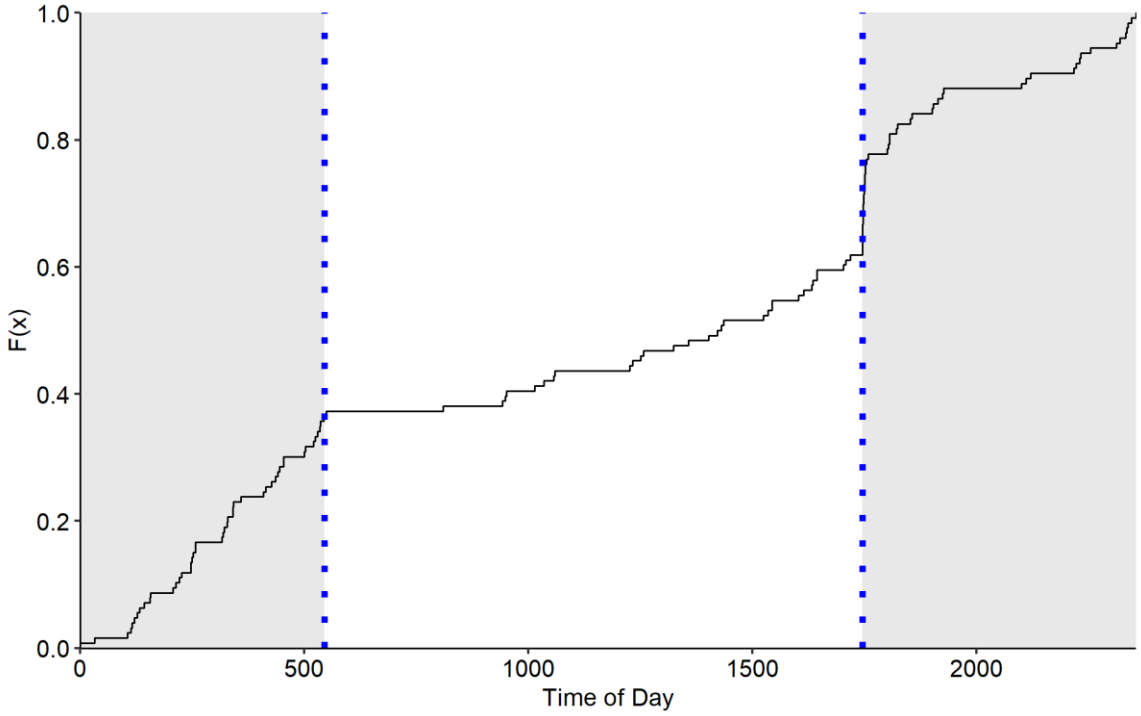


Figure 2-15. An empirical cumulative distribution function (ECDF) illustrating all 126 trials that resulted in a juvenile Flathead Catfish consuming a Goldfish and the time of day that consumption events occurred most frequently (steeper slope). Dashed blue lines indicate lights on (0545) and lights off (1745) in the laboratory.

CHAPTER 3: THE EFFECTS OF TEMPERATURE ON ABSORPTION EFFICIENCIES OF FLATHEAD CATFISH *PLYODICIS OLIVARIS*

ABSTRACT

Abiotic factors heavily influence feeding rates and energy budgets of aquatic organisms as well as the trophic dynamics occurring across aquatic ecosystems. Identifying the link between temperature and absorption efficiency is crucial in understanding how the environment influences physiological processes of a species. Freshwater species in temperate regions encounter a broad range of temperatures across life history stages, but the mechanistic link between temperature and metabolism often remains poorly understood, particularly for understudied species such as Flathead Catfish *Polydictis olivaris*. Understanding the effect of temperature on absorption efficiencies of an apex predator provides managers with insight into energy needs and energy allocations of the organism. Quantifying energy conversion at a species-specific level provides insight into phenotypic plasticity of an organism which may explain competitive advantages. Therefore, we examined the relationship between temperature and absorption efficiency of juvenile Flathead Catfish fed a single ration. We examined the caloric content remaining in the stomach and intestines of 126 individuals at three temperatures (17° C, 22° C, 25° C) and nine time intervals post-consumption. A bomb calorimeter was used to measure gross energy remaining of digested content. Temperature did not significantly change absorption efficiencies within

the stomach of juvenile Flathead Catfish, but did within the intestines. Furthermore, we tracked gross potential absorption of the whole meal throughout the alimentary canal and found there was no differences among temperatures. The ability of Juvenile Flathead Catfish to efficiently absorb nutrients throughout a range of temperatures may explain why growth rates are high across their distribution. Insight into absorption efficiencies provides managers with a better understanding of the bioenergetics of juvenile Flathead Catfish.

INTRODUCTION

Energy is the currency of all life (Rombourough 2006). Energy absorption is therefore an essential process in survival (i.e., promoting somatic and gonadal growth), and like most animals, fish acquire energy primarily from ingesting food (Eddy and Handy 2012). Brody (1945) suggests food intake, digestion, absorption, and conversion are the successive steps in which food is transformed into growth. One of the main principals explored in fish nutrition is that food must remain in the gut for an appropriate amount of time to be digested and for energy to be absorbed (Eddy and Handy 2012). Gut motility (e.g., gastric evacuation) is known to change in regard to fish size (Andersen 1999), type of prey item (Windell and Norris 1969), prey size (Legler et al. 2010), and temperature (Perrson 1979; Jobling 1980; Nakagawa 2018). Temperature is thought to be the most influential factor regulating evacuation since temperature can influence the

digestive process in a variety of ways, including feeding rate, secretion rates of digestive fluids and enzymes, gastric intestinal motility, and rate of intestinal absorption (Kapoor et al. 1975). Multiple studies have reported that an increase in temperature results in an increase in evacuation up to a threshold, after which it typically diminishes (Chapter 2 of this thesis, Tyler 1970; Bernreuther et al. 2009; Andersen 2012).

Riverine fishes have been exposed to changing thermal regimes over the past century, which in some cases means an increase in water temperature related to warming air temperatures (Kaushal et al. 2010; van Vliet et al. 2011). Anthropogenic alterations to rivers (e.g., channelization, altering flow rates, deforestation, and hydroelectric technologies) further contribute to changes in thermal regimes (Anderson et al 2006; Wondzell et al. 2019), which ultimately govern biological processes (e.g., species distribution) (Benyahya et al. 2010). Water temperature is one of the most important physical properties of a river, often controlling other physical aspects (e.g., dissolved oxygen levels; Harvey 2013), while having a direct impact on consumption rates and growth of fishes (Rose 2009; Krieger et al. 2019).

Environmental conditions (e.g., temperature) experienced by individuals result in differences in efficiency of conversion of food into growth (Brody 1945; Hanson et al. 1997; Deslauriers et al. 2017). Richman (1958) defines the efficiency of food conversion in two ways: gross efficiency of food conversion as the percentage of food consumed and net efficiency of conversion as the percent

of food absorbed. Digestion does not necessarily result in absorption (Gerking 1952); however, digestion is necessary in order for absorption to occur.

Prior to absorption occurring, food must be mechanically and enzymatically broken down into small enough molecules, a process known as digestion (Patricia and Dhamoon 2020). Digestion begins at the mouth where most predacious fish consume prey whole (Parenti and Weitzman 2019). From the mouth food is passed through the esophagus to the stomach. Digestion within the stomach occurs as a gradual inward process where layers of the consumed meal are reduced and transported to the intestines (Kapoor et al. 1975).

The physical passage of food through the alimentary canal varies by species and food types along with abiotic environmental factors. Digestion is a progressive process not ending until food leaves the rectum as waste (Smith 1980). The physical state of food changes form as it is passed along the alimentary canal, presumably to aid in absorption. Mucus, acid, and enzymes within the stomach breakdown the solid state of the food forming a liquid as it passes through the midgut before it solidifies forming feces in the hindgut (Smith 1980).

Morphology of fish intestines and stomach are indicative of the diet. Herbivorous fishes tend to have longer intestinal tracts to assist with the absorption of plant matter, while piscivorous fishes tend to have shorter intestines (Kramer and Bryant 1995). The intestines of all fish are an undifferentiated tube with no separation between small and large intestines (Canen et al. 2012).

Regardless of morphology, absorption has been found to occur primarily in the midgut of the intestines where the physical state of the food is in a liquefied form (Smith 1980). Some absorption is likely to occur in the stomach and hindgut; however, this has been minimally studied in fishes (Smith 1980).

Flathead Catfish *Plyodictis olivaris* are an endemic North American fish ranging west of the Appalachian Mountains throughout the Mississippi River Basin (Hrabik et al. 2015), as well as the Mobile and Rio Grande River Basins. Flathead Catfish have dispersed through natural range expansion and unauthorized introduction in many systems east of the Appalachians and west of the Rocky Mountains (Fuller et al. 1999). Their distribution highlights the plasticity of this species, with a wide range of thermal regimes they may experience not only spatially but also seasonally.

Flathead Catfish diets shifts from macroinvertebrates to piscivory as an adult (Minckley and Deacon 1959; Jackson 1999; Brewster 2007; Hogberg and Pegg 2011). The exact shift-at-size depends heavily on the food availability within the environment (Minckley and Deacon 1959, Brewster 2007, Hogberg and Pegg 2011). Flathead Catfish do not show differences in diet composition between seasons and are considered to be opportunistic feeders, primarily feeding on what is readily available within their microhabitat (e.g., Common Carp *Cyprinus carpio*, Flathead Catfish, and Shovelnose Sturgeon *Scaphirhynchus platorynchus*, as well as macroinvertebrate taxa Ephemeroptera and Plecoptera; Turner 2017). Turner (2017) found that consumption and stomach fullness rates

were similar between introduced and native populations. Temperature has been empirically shown to influence gastric evacuation rates of juvenile Flathead Catfish (Chapter 2 of this thesis), while little is known in regard to the effect of temperature on absorption efficiency.

Knowledge surrounding fish nutritional needs often comes from the few heavily cultured species in aquaculture (e.g., Salmon, Trout, Tilapia, and Carp). When trying to understand nutritional needs of an understudied species, scientists rely on stomach samples of wild caught fish (Eddy and Handy 2012). Stomach samples provide insight on diet composition, diet selectivity, and prey nutritional values which are often key components in understanding the quantity or quality of available food types. Empirical laboratory studies can pinpoint the energetic content remaining in the stomach and the intestines, thereby allow us to quantify how much energy has already been absorbed by the individual at known times post consumption. Laboratory experiments provide us with the unique capability of controlling abiotic factors and we can actively select individuals that are physiologically similar to better understand absorption.

Understanding the specific link between temperature and energy absorption of juvenile Flathead Catfish provides managers with insight into energy available toward fish growth during a crucial life-history stage. During the juvenile life-history stage, production is prioritized with somatic growth of the body and the functioning systems that sustain life of the individual (Wootton and Smith 2015). Eventually, adulthood results in a more complex allocation of

energy toward reproduction at a cost to somatic growth (Rijnsdorp 1990; Wootton and Smith 2015).

Few bioenergetics studies have been explored specifically for Flathead Catfish (e.g., Roell and Orth 1993; Hedden et al. 2016). The objective of this study was to address a gap in knowledge of how temperature effects absorption efficiencies of juvenile Flathead Catfish. We predicted that colder temperatures would result in more thorough energy absorption of a single ration due to a decreased gastric evacuation rate. As temperatures increases and gastric evacuation rate increases, we predicted absorption efficiency would decrease. This information will help biologist understand energy flow of a meal through Flathead Catfish at varying temperatures. Understanding temperature impacts on an individual fish can be scaled to a population level, and inferences can be made about population consumption and trophic dynamics.

METHODS

Data Collection

Juvenile Flathead Catfish were fed a single meal of Goldfish of known wet weight to the nearest 0.01 g. Juvenile Flathead Catfish stomach and intestinal content were dissected at nine different time intervals (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h) across three treatment temperatures of 17° C, 22° C, and 25° C. Dissection time intervals were selected based on previous gastric evacuation

rate studies (Ling and Ghaffar 2014). Temperatures were selected based on past studies, typical field conditions, and laboratory limitations. Bourret et al. (2008) determined that Flathead Catfish rarely ate below 15° C, while maximum daily consumption was significantly higher at 19° C and remained elevated until 32° C. Furthermore, yearly Missouri River temperatures range between 15° C to 30° C from May to October, peaking in mid-July (USGS 2019).

Food remaining in the esophagus and stomach at time of dissection was considered to be part of the stomach contents while food in the intestines and anus were considered to be part of the intestinal contents. Digested content was extracted by gently squeezing along the stomach and intestines. Dissection scissors were used to cut open the stomach to ensure all contents were collected. Digested matter was collected and saved for bomb calorimetry analysis. Vials containing digested content were placed in a -80° C freezer until all trials were completed. Laboratory setup and rearing procedures of Flathead Catfish can be found in Appendix 1 of this thesis. Collection, acclimation, and experimental setup can be found in Chapter 2 of this thesis.

Drying process of samples

Vials were removed from the -80°C freezer in groups of 40 to prevent excessive thawing. Metal weighing tins were marked with a unique identifier (UID) to track samples. Empty tins were weighed to the nearest 0.001 g and recorded. Deionized water was used to ensure all intestinal contents from within

the sample vial were emptied as thoroughly as possible. Tins were re-weighed with the contents to the nearest 0.001 g. We assumed the amount of deionized water used was negligible due to the evaporation of all moisture within each sample during the drying process.

Hyslop (1980) reviewed studies that dried stomach samples and found that temperatures greater than 80° C resulted in the loss of volatile lipids. Therefore, we selected a drying temperature of 70° C, using an Isotemp drying oven (Fisher Scientific, Waltham, Massachusetts, U.S.A.). Tin and contents were reweighed every 24 hours until the combined weight of the tin and contents did not vary by more than 0.01g since the previous 24-hour weighing period. Drying time ranged between 48-72 hours for all samples. The initial tin weight was subtracted from the final combined weight to determine final dry weight (DW) of the sample. A scalpel was used to scrape dried contents from the tin into a new vial marked with the appropriate UID. Dried samples were stored under a fume hood until all samples were ready for bomb calorimetry. A subsample of dried contents was reweighed at the beginning of bombing to ensure that moisture had not reentered the samples.

Bomb calorimetry of samples

Dried stomach and intestine contents were analyzed using a Parr 1425 semi-micro bomb calorimeter (Parr Instruments, Moline, Illinois, U.S.A.). This calorimeter is capable of combusting pelleted samples between 0.025 and 0.200 g

DW. Samples that contained more than 0.075 g DW were separated into three subsamples. Two subsamples were analyzed in the bomb calorimeter, and the third subsample was analyzed only if the caloric values from the first two samples varied by more than 10%. If a sample weighed less than 0.075 g DW, the sample was divided evenly and the two subsamples were processed. If caloric values differed by more than 10%, the data were excluded from our analysis. All samples were pressed into ¼ inch diameter pellets using a Parr 2812 Pellet Press (Parr Instruments, Moline, Illinois, U.S.A.) prior to analysis in the bomb calorimeter.

To perform calorimetry, a 10 cm length of aluminum fuse wire was attached to the 1107 Bomb (Parr Instruments, Moline, Illinois, U.S.A.) as described by the Parr 1425 operating instruction manual. A pressed pellet was placed within the 1107 Bomb so that it was touching the charge. The bomb was closed carefully ensuring that the sample and the charge remained in contact. Each bomb was filled with 30 to 35 atmospheres of pure oxygen and placed in the Dewar flask, which was filled with 450 milliliters of distilled water. All seals were replaced on the bomb at the beginning of our experiment to ensure no oxygen leaking occurred. If a leak was noticed by indication of bubbles in the Dewar flask, the bomb was dismantled, the charge was reset, and the bomb was refilled to the appropriate 30-35 atmospheres of pressure of oxygen.

Ignition wires were attached and a lid was placed over the Dewar flask. The lid was mounted with both a thermometer and a stirrer. The stirrer was activated and a pre-period trace was initiated by the thermometer to ensure that a

stable temperature was held prior to combustion. The machine would indicate when temperature equilibrium had been achieved, at which point the charge was fired and a distinct temperature change was detected. The trace continued until the recorded temperature reached an asymptote. The bomb was disassembled to ensure the sample and wire were combusted completely. Any adjustments were calculated and recorded for each combustion of a sample. Caloric content was adjusted based on the amount of unburnt wire after combustion to account for any potential errors. A correction of 1400 calories per 1.0 g of unburned wire was used. Corrections for nitric and sulfuric acids are typically not significant for semi-micro samples and we assumed them to be negligible. Final caloric values generated from the bomb calorimeter were measured in calories per gram DW.

Predicting amount of calories initially fed

We estimated the initial calories fed to our Flathead Catfish by assessing the caloric content of a subsample of Goldfish (n = 28). We euthanized Goldfish in a MS-222 solution and measured their wet weight (WW) to the nearest 0.01g. We dried the Goldfish at 70º C for a total of 72 hours, reweighing them every 24 hours. Weights of individual Goldfish did not differ by more than 0.01 g between the 48th and 72nd hour. A linear regression was used to model the relationship between WW and DW of our Goldfish subsample. The equation of the regression line allowed us to predict the DW of all consumed Goldfish based on their known WW.

The average caloric value per g DW of Goldfish was calculated by quantifying the caloric content of a subsample of Goldfish (n = 17). Individual Goldfish were homogenized and pressed into ¼ inch pellets and analyzed in the same manner as stomach and intestinal contents. The average value from the subsample of analyzed Goldfish was multiplied by the predicted DW of each ration to estimate initial calories consumed.

Data Analysis

Generalized linear models were used to evaluate the effect that digestion time, temperature, ration size, predator size, age, sex, and experimental tank had on the rate of absorption in the digestive system of juvenile Flathead Catfish. We used the corrected Akaike's Information Criterion (AICc) to assess model fit. If competing models existed in explaining calories/g DW (e.g., within 2.0 delta AIC from the best-fit model), we chose the simplest model (e.g., the model with the fewest parameters; K).

We first examined the calories/g DW remaining through time in the stomach and then the intestines. We performed a Shapiro-Wilk test to assess normality of our data and ultimately log transformed calories/g DW data from the intestines. We constructed general linear models (family Gaussian) to predict factors effecting calorie absorption. We analyzed our best-fit models using a Chi-square test to determine which terms were significant. Whenever ration size was a significant model predictor, it was held constant with its mean value of 0.015. A

post-hoc test was used if treatment temperature was significant within our model. We used least square means for multiple comparison with a Tukey-adjustment of p-values to test where differences were present between treatments (Lenth and Hervé 2015).

We also examined the potential gross absorption of the ration through time. We calculated the calories remaining in the stomach of an individual by multiplying the DW of the stomach content by the calories/g DW of the stomach content. We then calculated the calories remaining in the intestines of an individual by multiplying the DW of the intestines content by calories/g DW of the intestine sample. We combined the data of calories remaining within the stomach and intestines for each Flathead Catfish. We calculated the gross absorption of calories as the difference between the predicted calories consumed and the sum of the calories remaining. We standardized our data by creating a proportion of calories absorbed, which was done by dividing the predicted calories consumed by the gross absorbed calories.

We assumed the proportion of calories absorbed to be bound between values of 0 to 1. Data were non-normally distributed due to the use of proportions. Logit transformations are appropriate when stabilizing variance of proportions and percentages in binomial distributions (Wilson et al. 2013). Therefore, we ran binomial logistic regression on the proportion of calories absorbed to determine if there was a difference among treatment temperatures. Binomial regression output is in units of logits, which were transformed into odds with:

$$odds = \exp(logits)$$

and then a proportion with:

$$proportion = odds/(1 + odds).$$

We created a predictive model to illustrate the proportion of gross potential absorption occurring through time and by treatment.

Understanding that gastric digestion occurs in layers with a majority of absorption occurring within the intestines (Smith 1990), we specifically examined time points Flathead Catfish were still actively evacuating their meal from their stomachs. Juvenile Flathead Catfish stomachs were empty at 19 hours 39 minutes, 16 hours 9 minutes, and 14 hours 33 minutes at 17° C, 22° C, and 25° C (Chapter 2 of this thesis). Therefore, we examined gross potential absorption occurring between 2 to 16 hours post consumption for our three treatments. Significant differences were determined based on $\alpha = 0.05$. All statistical analysis were performed using R version 3.5.2.

RESULTS

Estimate of ration dry weight and calories

We found a strong linear relationship between WW and DW of our Goldfish subsample ($n = 28$, $F_{1,26} = 939.360$, $P < 0.001$; Figure 3-1). We calculated predicted DW of a ration by the equation

$$\hat{y} = -0.04 + 0.19x$$

where $\hat{y}$ is the predicted initial DW of the ration and x is the ration WW.

No relationship existed between Goldfish DW and calories per gram DW of our Goldfish subsample (n = 17, $F_{1,15} = 0.728$, P = 0.407; Figure 3-2), suggesting that caloric content per one gram DW remained similar across the prey size range. The mean caloric value of all bombed Goldfish was 3852.50 calories/g DW (Table A3-1). We calculated predicted calories of a ration by the equation

$$\hat{y} = 3852.50x$$

where $\hat{y}$ is the predicted initial calories of the ration and x is the predicted ration DW.

Stomach and intestine caloric content remaining

We quantified caloric value of the stomach contents of 67 juvenile Flathead Catfish; the remaining 59 individuals had empty stomachs at the time of digestion observed. Of the 67 Flathead Catfish, 10 of the initial two bombings varied by more than 10% in the caloric values of the samples. Therefore, we incorporated a third measurement of the contents to ensure variability was reduced. Three Flathead Catfish only had enough stomach contents (< 0.025 g DW) to perform a measurement once.

The raw data of calories/g DW remaining in the stomach did not differ significantly from a normal distribution (W = 0.965, P = 0.055). The calories/g DW remaining in stomach contents can be best explained by digestion time and ration size (Table 3-1; Figure 3-3). Temperature was not a significant predictor of

calories/g DW remaining in the stomachs of juvenile Flathead Catfish. The predicted regression equation using our best-fit model was:

$$y' = 4094.320 - 41.890(t) + 19600.110(Rs),$$

where *y`* = the predicted calories/g DW remaining in the stomach, *t* = digestion time, and *Rs* = the ration size. Based on our model, 4305 calories/g DW remained in the stomach content of juvenile Flathead Catfish at hour 2 compared to 3718 calories/g DW at hour 16. The model predicted a decline of 42 calories/g DW in stomach content every hour.

We measured the caloric value of intestinal contents of 111 juvenile Flathead Catfish; the remaining 15 individuals had empty intestines at the time of digestion observed. Of the 111 bombed intestinal samples, 13 of the initial two bombings varied by more than 10% in the caloric values of the sample. Therefore, we incorporated a third bombing of the contents to ensure variability was reduced. Twenty-five Flathead Catfish only had enough intestinal content (< 0.025 g DW) to perform a measurement once.

We removed 15 individuals from our dataset that had an observed zero calories remaining within the intestines to assess the effects of temperature on the calories/g DW through time. Zero calories/g DW were noticed at both early and later time points throughout all three treatment temperatures. Removing zero calorie intestinal data did not result in a normal distribution ($W = 0.930$, $P < 0.001$). Therefore, we log transformed the intestinal data, which resulted in a non-significant difference from a normal distribution ($W = 0.988$, $P = 0.414$).

Log (calories/g DW) remaining in intestinal content can be best explained by three models (Table 3-2). We used the simplest model, which accounted for digestion time and temperature as the predictors of calories/g DW remaining in the intestines. There was a significant difference (χ^2 = 24.239, df = 2, P < .001; Table 3-3) in calories/g DW remaining in the intestines among treatments. A post-hoc analysis found differences in log (calories/g DW) remaining in intestinal content between treatments of 17º C and 25º C (P < 0.001) as well as 22º C and 25º C (P = 0.012), but not between 17º C and 22º C (P = 0.133; Table 3-4). The predicted regression equation using our best-fit model was:

$$y' = 3.462 - 0.005(t) - 0.040(T_{22}) - 0.093(T_{25}),$$

where *y`* = the predicted calories/g DW remaining in the intestines as a common logarithm, *t* = digestion time, T_{22} = binary input (0 or 1) used t to examine 22° C fish, and T_{25} = binary input (0 or 1) used to examine 25° C fish. A back transformation was performed on our logged data to illustrate the change in calories/g DW within the intestines on the original units (Figure 3-4).

Total calories absorbed

The sum of the calories remaining in the stomach and the intestines provided the total caloric value remaining in the alimentary canal. Eleven individuals had higher caloric values remaining in the digestive tract than the predicted initial caloric value of the ration. All 11 Flathead Catfish were assessed at early digestion times (2 hours, 7 individuals; 4 hours, 3 individuals; and 6

hours, 1 individual) and among all three treatments (17º C, 3 individuals; 22º C, 3 individuals; and 25º C, 5 individuals). We omitted these 11 individuals from our analysis of potential gross absorption.

We found the proportion of gross calories absorbed through the entire alimentary canal was best explained by two models (Table 3-5). The simplest model accounted for digestion time, temperature, and ration size. There was no difference (χ^2 = 4.607, df = 2, P = 0.100; Table 3-6) in the proportion of gross calorie absorption among treatments (Figure 3-5). The predicted regression equation using our best-fit model was:

$$PropAborb` = -1.465 + 0.116(t) + 0.939(T_{22}) + 1.219(T_{25}) - 36.416(Rs),$$

where PropAbsorb` = predicted logits value of gross calories absorbed, t = digestion time, T_{22} = binary input (0 or 1) used to examine 22°C fish, T_{25} = binary input (0 or 1) used to examine 25°C, and Rs = ration size.

Additionally, the proportion of gross calorie absorption was not different among temperature treatments when confining datum between 2 to 16 hours post consumption (χ^2 = 3.34, df = 2, P = 0.188; Table 3-7; Figure 3-6). The predicted regression equation using our best-fit model was:

$$RestictedPropAbsorb` = -1.969 + 0.200(t) + 0.905(T_{22}) + 1.201(T_{25}) - 48.956(Rs),$$

where $RestictedPropAbsorb$ ` = predicted logits value of gross calories absorbed within our restricted range of 2 to 16 hours post consumption, t =

digestion time, T_{22} = binary input (0 or 1) used to examine 22°C fish, T_{25} = binary input (0 or 1) used to examine 25°C, and Rs = ration size.

DISCUSSION

Quantitative estimates of energy transformation are a useful approach when trying to understand the growth of an organism. Temperature is known to influence the rates of nearly all biological functions of fishes (Jobling 1997). Alterations in an organism's ability to absorb nutrients inevitably influences conversion of food into growth. Our research helps fill the knowledge gap of the effects of temperature on the potential for gross absorption of an apex predator. We found that relatively little absorption occurred within the stomach of juvenile Flathead Catfish, while most of absorption took place within the first few hours following passage of food into the intestines. Furthermore, our results showed that potential gross absorption was similar among three temperature treatments following the ingestion of a single meal.

We found that the calories/g DW of content remaining in the stomachs of juvenile Flathead Catfish did not differ among our treatments. Time since consumption was a significant predictor within the model and indicated a decrease of 42 calories/g DW of stomach contents for every additional hour post consumption. The small hourly reduction in calorie content/g DW in the stomach could be due to a number of reasons. Digestion within the stomach usually occurs

in layers where a meal is reduced in size and prepared for absorption within the intestines (Kapoor et. al 1975). This suggests that easy to digest and energy-rich content (e.g., soft tissues and muscle) are broken down and passed into the intestines at earlier time points of digestion, leaving behind less energy-rich content (e.g., calcified structures such as bone and scales) from one time point to the next. Another possibility we observed a slight decrease of caloric content/g DW in the stomach is that some degree of absorption is occurring within the stomach, thus reducing the caloric content per gram. However, previous literature suggests that this has been minimally studied and that most absorption takes place within the intestines of fishes (Smith 1980).

We found differences in the amount of calories/g DW remaining in the intestines between 17º C and 25º C as well as 22º C and 25º C. The highest absolute values of caloric content being depleted from food (i.e., assumed to be absorbed) were observed in fish in the warmest water and at the earliest time points. We observed digested food enter the intestines as early as 2 hours post consumption, with an estimated caloric range of 2800 to 4600 calories/g DW. The elevated calories/g DW observed at hour 2 within the intestines validated the hypothesis that nutrient-rich content was being passed from the stomach at earlier time points. The calories/g DW quickly decreased from hour 2 to hour 4 within the intestines, suggesting a large amount of absorption occurred between these time intervals. We anecdotally observed food within the mid gut (e.g., upper intestines) was in a liquid form before re-solidifying as it approached the anus,

supporting the results of Smith (1980). During the liquid stage, it was likely that food in contact with the intestinal wall was most easily assimilated into the blood stream.

The gross potential of absorption remained similar among treatment temperatures. This suggests that juvenile Flathead Catfish fed a single ration are capable of absorbing a similar maximum amount of calories from the food source regardless of temperature. This finding is surprising, given that we predicted potential energy to be lost at warmer temperatures due to faster evacuation rates and lower absorption efficiencies. Based on the observed differences of the effect of temperature on the calories/g DW remaining in the intestines, it seemed likely that absorption efficiencies increased in warmer temperature fish to compensate for faster passage of the meal along the entire digestive tract, though the mechanism mediating this potential compensation remains unknown.

It is important to note that we quantified potential gross absorption using the average calories/g DW remaining within the entire intestinal tract. When calculating the calories/g DW remaining within the intestines we did not distinguish between sections such as the mid-gut or hind-gut. Sub-dividing the intestines in future work could provide greater resolution of energy absorption within the intestines of juvenile Flathead Catfish. We found caloric content within the stomach remained similar (approximately 4000 calories/g DW) among the observed time points that food was still present in the stomach (2 to 24 hours). With the exception of hour 2, we observed caloric content within the intestines

was much lower than 4000 calories/g DW (range = 1302.60 – 3307.48 calories/g DW; mean = 2243.82 calories/g DW). The process of homogenizing samples that may have just entered the intestines with content that had been catabolizing for a period of time could have neutralized the caloric values at either end of the intestines.

Shorter time interval observations of energy content remaining in the intestines could provide useful information in determining energy absorption for Flathead Catfish. Furthermore, we found intestinal contents in some individuals even at our longest time point post-consumption of 48 hours. We suggest assessing digestion in 30-minute intervals during the first couple of hours of digestion and extending time points beyond 48 hours, especially at temperatures below 17º C.

Understanding the effects of temperature on energy absorption of a top predator is particularly useful for managers looking to quantify energy flow within a food web, and how energy is converted into growth. Flathead Catfish are one of the fastest growing freshwater fishes in North America (Jackson 1999), yet little research exists in quantifying energy absorption. Our results support the conclusions of Bourret et al. (2008) suggesting that the range of temperatures at which Flathead Catfish can feed at an elevated rate could account for the documented fast growth rates. Although our study evaluated a limited range in temperatures, our findings suggest plasticity in an individual's ability to absorb nutrients. Plasticity in absorption of nutrients could be beneficial to growth rates

and survival of this species by elongated periods of growth. Continued growth of juvenile Flathead Catfish across a large range of temperatures can facilitate increases in condition, survival, reproduction, and ultimately, population growth over those of its competitors.

Understanding the bioenergetics of apex predators provides managers with insight on how warming waters due to climate change may impact fish populations due to changes in physiology, or alteration in biotic interactions such as predation. Predation is an important driver in the community structure of fishes (Boaden and Kingsford 2015). As temperature increases, consumption rates of predators will need to increase to match elevated energetic demands due to higher routine metabolic rates and somatic and gonadal growth. Increasing temperatures also affect habitat availability for both predators and prey. Shifting distributions may alter trophic dynamics and species assemblages. This may then impact the ability of predators to compensate for increased consumption rates, and force them to increase the size and scope of their native range. For apex predators in particular, range expansion poses a serious risk to systems where they have been historically absent. Flathead Catfish are one of the least gape-limited piscivores (Slaughter and Jacobson 2008) and introduced individuals have been found to cause native fish populations to decline and shifts in intraspecific competition (Kwak et al. 2006).

Our results support the findings of Kapoor et al. (1975), suggesting that temperature can influence the digestive and absorptive processes of fishes in a

variety of ways. These include the effects of temperature on feeding rates (Chapter 2 of this thesis), secretion rates of digestive fluids and enzymes (anecdotally observed), gastric evacuation rates (Chapter 2 of this thesis), and the rate of intestinal absorption (as seen in juvenile Flathead Catfish). Measuring these effects can be quite challenging as the effects of temperature are multifaceted. However, our experiment lays the groundwork examining the influence of temperature on absorption efficiencies of a top predator.

LITERATURE CITED

Andersen, N. G. 1999. The effects of predator size, temperature, and prey characteristics on gastric evacuation in Whiting. Journal of Fish Biology 54(2):287–301.

Andersen, N. G. 2012. Influences of potential predictor variables on gastric evacuation in Atlantic Cod *Gadus morhua* feeding on fish prey: parameterization of a generic model. Journal of Fish Biology 80(3):595–612.

Anderson, E. P., M. C. Freeman, and C. M. Pringle. 2006. Ecological consequences of hydropower development in Central America: Impacts of small dams and water diversion on neotropical stream fish assemblages. River Research and Applications 22(4):397–411.

Benyahya, L., A. St-Hilaire, T. B. M. J. Ouarda, B. Bobée, and J. Dumas. 2008. Comparison of non-parametric and parametric water temperature models on the Nivelle River, France. Hydrological Sciences Journal 53(3):640–655.

Bernreuther, M., A. Temming, and J. P. Herrmann. 2009. Effect of temperature on the gastric evacuation in Sprat *Sprattus sprattus*. Journal of Fish Biology 75(7):1525–1541.

Boaden, A. E., and M. J. Kingsford. 2015. Predators drive community structure in coral reef fish assemblages. Ecosphere 6(4):1–33.

Bourret, S. L., R. W. Tingley, Y. Kanno, and J. C. Vokoun. 2008. Maximum daily consumption and specific daily metabolic demand of juvenile Flathead Catfish *Pylodictis olivaris*. Journal of Freshwater Ecology 23(3):413–419.

Brewster J. R. 2007. Trophic relations of introduced Flathead Catfish in a North Carolina Piedmont River. Master's thesis. North Carolina State University, Raleigh.

Brody, S. 1945. Bioenergetics and growth. Reinhold Publishing, New York.

Brown, J. C. 2003. Energy, Measurement of food energy. Page 2087 *In* B. Caballero, L. C. Trugo, and P. M. Finglas, editors. Encyclopedia of Food Sciences and Nutrition, Second Edition. Academic Press.

Deslauriers, D., S. R. Chipps, J. E. Breck, J. A. Rice, and C. P. Madenjian. 2017. Fish bioenergetics 4.0: an R-based modeling application. Fisheries 42(11):586–596.

Eddy, F. B., and R. D. Handy. 2012. Ecological and environmental physiology of fishes. Oxford University Press. Oxford, United Kingdom.

Gerking, S. D. 1952. The protein metabolism of sunfishes of different ages. Physiological Zoology 25(4):358-372.

Hanson, P. C., T. B. Johnson, D. E. Schindler and J. F. Kitchell. 1997. Fish bioenergetics 3.0 Madison: University of Wisconsin Sea Grant Institute. Report WISCU-T-97–001.

Harvey, R., L. Lye, A. Khan, and R. Paterson. 2011. The influence of air temperature on water temperature and the concentration of dissolved oxygen in Newfoundland Rivers. Canadian Water Resources Journal 36(2):171–192.

Hedden, S. C., K. B. Gido, and J. E. Whitney. 2016. Introduced Flathead Catfish consumptive demand on native fishes of the upper Gila River, New Mexico. North American Journal of Fisheries Management 36(1):55–61.

Hogberg, N. P., and M. A. Pegg. 2016. Flathead Catfish *Pylodictis olivaris* diet composition during extreme flow events in a large river. Journal of Freshwater Ecology 31(3):431–441.

Hrabik, R.A., S.C. Schainost, R.H. Stasiak, and E.J. Peters. 2015. The fishes of Nebraska. Mennonite Press, Newton, Kansas.

Hyslop, E. J. 1980. Stomach contents analysis-a review of methods and their application. Journal of Fish Biology 17(4):411–429.

Jackson, D. C. 1999. Flathead Catfish: biology, fisheries, and management. Catfish 2000: proceedings of the international ictalurid symposium. American Fisheries Society, Symposium 24, Bethesda, Maryland.

Jobling, M. 1980. Gastric evacuation in Plaice, *Pleuronectes platessa*: effects of temperature and fish size. Journal of Fish Biology 17:547-551.

Jobling, M. 1997. Temperature and growth: modulation of growth rate via temperature change. Pages 225–254 *in* C. M. Wood and D. G. McDonald,

editors. Global Warming: Implications for Freshwater and Marine Fish. Cambridge University Press, Cambridge.

Kapoor, B.G., H. Smit, and I. A. Verighina. 1975. Digestion rate. Pages 180-188 *In* F. S. Russell and M. Yonge, editors, Advances in Marine Biology (pp. 180-188). Academic Press, New York.

Kaushal, S. S., G. E. Likens, N. A. Jaworski, M. L. Pace, A. M. Sides, D. Seekell, K. T. Belt, D. H. Secor, and R. L. Wingate. 2010. Rising stream and river temperatures in the United States. Frontiers in Ecology and the Environment 8(9):461–466.

Kramer, D. L., and M. J. Bryant. 1995. Intestine length in the fishes of a tropical stream: 2. Relationships to diet — the long and short of a convoluted issue. Environmental Biology of Fishes 42(2):129–141.

Krieger, J. R., A. Sreenivasan, and R. Heintz. 2019. Temperature-dependent growth and consumption of young-of-the-year sablefish *Anoplopoma fimbria*: Too hot, too cold or just right? Fisheries Research 209:32–39.

Kwak, T. J., D. S. Waters, and W. E. Pine. 2006. Age, growth, and mortality of introduced Flathead Catfish in Atlantic rivers and a review of other populations. North American Journal of Fisheries Management 26(1):73–87.

Legler, N. D., T. B. Johnson, D. D. Heath, and S. A. Ludsin. 2010. Water temperature and prey size effects on the rate of digestion of larval and early juvenile fish. Transactions of the American Fisheries Society 139(3):868–875.

Lenth, R.V. and M. Hervé. 2015. Package 'lsmeans'. Available: cran.r-project.org/web/packages/lsmeans/lsmeans.pdf

Ling, K. M., and M. A. Ghaffar. 2014. Estimation of gastric emptying time (GET) in Clownfish *Amphiprion ocellaris* using x-radiography technique. *In* AIP Conference Proceedings 1614:624-628. Available: https://aip.scitation.org/doi/abs/10.1063/1.4895275

Minckley, W. L., and J. E. Deacon. 1959. Biology of the Flathead Catfish in Kansas. Transactions of the American Fisheries Society 88(4):344-355.

Nakagawa, H. 2018. Temperature-dependent gastric evacuation rate of the Japanese Delicate Loach *Niwaella delicata* (Cobitidae). Ichthyological Research 65(1):172–174.

Parenti, L. R., and S.H. Weitzman. 2019. Fish *In* Encyclopædia Britannica. Encyclopædia Britannica. Available: https://www.britannica.com/animal/fish

Patricia J.J., and A.S. Dhamoon. 2020. Physiology, Digestion *In*: StatPearls. StatPearls Publishing, Treasure Island, Florida. Available: https://www.ncbi.nlm.nih.gov/books/NBK544242/

Perrson, L. 1979. The effects of temperature and different food organisms on the rate of gastric evacuation in perch (*Perca fluviatilis*). Freshwater Biology 9:99-104.

Richman, S. 1958. The Transformation of Energy by *Daphnia pulex*. Ecological Monographs, 28(3):273–291.

Rijnsdorp, A. D. 1990. The mechanism of energy allocation over reproduction and somatic growth in female North Sea plaice, *Pleuronectes platessa*. Netherlands Journal of Sea Research *25*(1-2): 279-289.

Roell, M. J., and D. J. Orth. 1993. Trophic basis of production of stream-dwelling Smallmouth Bass, Rock Bass, and Flathead Catfish in relation to invertebrate bait harvest. Transactions of the American Fisheries Society 122(1):46–62.

Rose, J., N. M. Vora, P. D. Countway, R. J. Gast, and D. A. Caron. 2009. Effects of temperature on growth rate and gross growth efficiency of an Antarctic bacterivorous protist. Multidisciplinary Journal of Microbial Ecology 3:252–260.

Slaughter, J. E., and B. Jacobson. 2008. Gape: body size relationship of Flathead Catfish. North American Journal of Fisheries Management 28(1):198–202

Smith, R. R. 1980. Nutritional bioenergetics in fish. *In* K. W. Chow, editor. Fish Feed Technology. FAO (Food and Agriculture Organization of the United Nations), ADCP/REP/80/11, Rome. Available: http://www.fao.org/3/x5738e/x5738e00.htm#Contents.

Turner, D. R. 2017. Trophic dynamics of Flathead Catfish in the Missouri River bordering Nebraska. Master's thesis. University of Nebraska, Lincoln.

Tyler, A. V. 1970. Rates of gastric emptying in young cod. Journal of the Fisheries Research Board of Canada 27(7):1177–1189.

USGS (United States Geological Survey). 2019. USGS water data for the nation [online database]. United States Geological Survey, Reston, Virginia. Available: https://waterdata.usgs.gov/usa/nwis/uv?site_no=06807000

van Vliet, M. T. H., F. Ludwig, J. J. G. Zwolsman, G. P. Weedon, and P. Kabat. 2011. Global river temperatures and sensitivity to atmospheric warming and changes in river flow: Sensitivity of global river temperatures. Water Resources Research 47(2):W02544.

Windell, J. T., and D. O. Norris. 1969. Gastric digestion and evacuation in Rainbow Trout. The Progressive Fish-Culturist 31(1):20–26.

Wilson, E., M. Underwood, O. Puckrin, K. Letto, R. Doyle, H. Caravan, S. Camus, and K. Bassett. 2013. The arcsine transformation: Has the time come for retirement? Available:https://www.mun.ca/biology/dschneider/b7932/B7932Final10Dec2010.pdf

Wondzell, S. M., M. Diabat, and R. Haggerty. 2019. What matters most: Are future stream temperatures more sensitive to changing air temperatures, discharge, or riparian vegetation? Journal of the American Water Resources Association 55(1):116–132.

Wootton, R. J., and C. Smith. 2015.Pages 109-110. *In* Reproductive biology of teleost fishes. Wiley-Blackwell, Oxford, UK.

Table 3-1. Generalized linear model (family Gaussian) selection, using Akaike information criterion (AICc), for calories/g dry weight remaining within the stomach of juvenile Flathead Catfish. We examined digestion time (hr), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), sex, Flathead Catfish length, and experimental tanks as predictors of the calories/g dry weight remaining in the stomachs of juvenile Flathead Catfish.

Model	K	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log-liklihood
Digestion Time + Ration Size	4	1070.64	0	0.53	0.53	-531.00
Ration Size	3	1072.74	2.1	0.18	0.71	-533.18
Digestion Time + Temperature + Ration Size	6	1074.15	3.52	0.09	0.8	-530.38
Null	2	1074.16	3.52	0.09	0.89	-534.98
Digestion Time	3	1074.99	4.35	0.06	0.95	-534.31
Digestion Time + Temperature + Ration Size + Sex + Tank + Flathead Catfish Length	10	1077.74	7.1	0.02	0.97	-526.91
Temperature	4	1077.92	7.28	0.01	0.98	-534.64
Digestion Time + Temperature	5	1078.61	7.97	0.01	0.99	-533.81
Digestion Time * Ration Size	11	1079.09	8.45	0.01	1	-526.14
Digestion Time * Temperature	12	1080.91	10.28	0	1	-525.57
Digestion Time * Temperature * Ration Size	17	1091.19	20.55	0	1	-522.35

Dependent variable: calories/g dry weight remaining within the stomach
K = number of parameters

Table 3-2. Generalized linear model (family Gaussian) selection, using Akaike information criterion (AICc), for calories/g dry weight remaining within the intestines of juvenile Flathead Catfish. We examined digestion time (hrs), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), sex, Flathead Catfish length, and experimental tanks as predictors of the calories/g dry weight remaining in the intestines of juvenile Flathead Catfish.

Model	K	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log-likelihood
Digestion Time + Temperature + Ration Size	6	-45.67	0	0.39	0.39	29.24
Digestion Time + Temperature	5	-45.13	0.53	0.3	0.69	27.86
Digestion Time + Temperature + Ration Size + Sex + Tank + Flathead Catfish Length	10	-43.82	1.84	0.16	0.85	33.02
Digestion Time * Temperature	12	-42.87	2.8	0.1	0.94	35.04
Digestion Time * Temperature * Ration Size	17	-41.77	3.9	0.06	1	41.21
Digestion Time + Ration Size	4	-27.26	18.41	0	1	17.82
Digestion Time	3	-26.83	18.84	0	1	16.53
Temperature	4	8.73	54.4	0	1	-0.17
Null	2	15.8	61.47	0	1	-5.84
Ration Size	3	16.07	61.74	0	1	-4.92

Dependent variable: Log calories/g DW remaining within the intestines
K = number of parameters

Table 3-3. Results of an ANOVA (α= 0.05) of digestion time (hr) and treatment temperature (17º C, 22º C, and 25º C) on the calories/g dry weight remaining in the intestines of juvenile Flathead Catfish.

	Df	Deviance	Residual Df	Residual Deviance	Pr(>Chi)
Null			109	7.16	
Digestion Time	1	2.39	108	4.77	< **0.001**
Temperature	2	0.89	106	3.88	< **0.001**

Dependent variable = Log (calories/g dry weight remaining in the intestines)
Df = degrees of freedom

Table 3-4. Results of a post-hoc analysis using least square means for multiple comparison (Tukey-adjusted comparison) to examine differences in calories/g dry weight remaining in the intestines of juvenile Flathead Catfish among treatments (17º C, 22º C, 25º C). Mean calories/g dry weight were different between treatments based on $\alpha = 0.05$.

	Estimate	**SE**	**Df**	**z-ratio**	**p-value**
17º C - 22º C	0.04	0.02	Inf	1.92	0.133
17º C - 25º C	0.9	0.02	Inf	4.84	**< 0.001**
22º C - 25º C	0.05	0.02	Inf	2.85	**0.012**

SE = standard error
Df = degrees of freedom

Table 3-5. Generalized linear model (family binomial) selection, using Akaike information criterion (AICc), for the proportion of gross calorie absorption throughout the entire alimentary canal of juvenile Flathead Catfish. We examined digestion time (hrs), temperature (º C), ration size (meal size as a proportion of Flathead Catfish body weight), experimental tank, sex, and age as predictors of the proportion of gross calorie absorbed.

Model	K	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log-liklihood
Digestion Time + Temperature + Ration Size	5	87.54	0	0.55	0.55	-38.49
Digestion Time + Temperature + Ration Size + Flathead Catfish Length	6	88.73	1.2	0.3	0.86	-37.98
Digestion Time + Ration Size	3	91.84	4.3	0.06	0.92	-42.81
Digestion Time + Temperature	4	93.68	6.14	0.03	0.95	-42.66
Digestion Time + Temperature + Flathead Catfish Length	5	93.85	6.31	0.02	0.97	-41.65
Digestion Time + Ration Size + Sex	5	94.53	6.99	0.02	0.99	-41.99
Digestion Time + Temperature + Ration Size + Sex + Age + Flathead Catfish Length + Tank	10	96.75	9.21	0.01	0.99	-37.29
Digestion Time	2	97.54	10	0	1	-46.71
Digestion Time * Temperature	6	98.36	10.82	0	1	-42.79
Flathead Catfish Length	2	146.92	59.38	0	1	-71.41
Temperature	3	147.71	60.18	0	1	-70.75
Null	1	148.52	60.99	0	1	-73.24
Tank	2	150.01	62.47	0	1	-72.95

Dependent variable: Proportion of absorbed calories

Table 3-6. Results of an ANOVA (α= 0.05) of digestion time (hr), treatment temperature (17º C, 22º C, and 25º C), and ration size (meal size as a proportion of Flathead Catfish body weight) on the proportion of gross calorie absorption (total calories assessed in stomach and intestines divided by initial predicted calories fed) of juvenile Flathead Catfish.

	Df	**Deviance**	**Residual Df**	**Residual Deviance**	**Pr(>Chi)**
Null			114	51.07	
Digestion Time	1	32.68	113	18.39	**< 0.001**
Temperature	2	4.41	111	13.98	0.110
Ration Size	1	2.48	110	11.51	0.116

Dependent variable = Proportion of absorbed calories.
Df = Degrees of freedom

Table 3-7. Results of an ANOVA (α= 0.05) of digestion time (hr), treatment temperature (17º C, 22º C, and 25º C), and ration size (meal size as a proportion of Flathead Catfish body weight) on the proportion of gross calorie absorption (total calories assessed in stomach and intestines divided by initial predicted calories fed) of juvenile Flathead Catfish restricted to 2-16 hours post consumption.

	Df	**Deviance**	**Residual Df**	**Residual Deviance**	**Pr(>Chi)**
Null			71	22.45	
Digestion Time	1	9.15	70	13.30	**< 0.001**
Temperature	2	3.35	68	9.95	0.188
Ration Size	1	3.44	67	6.51	0.064

Dependent variable = Proportion of absorbed calories
Df = Degrees of freedom

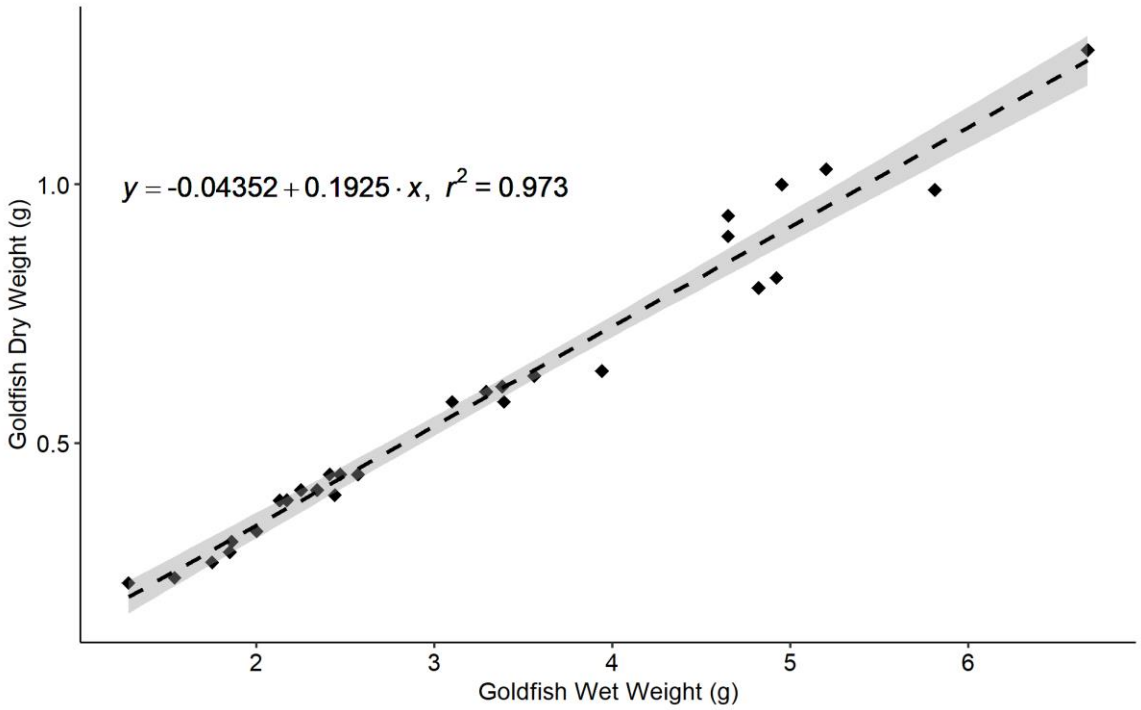


Figure 3-1. A simple linear regression showing the strong linear relationship between dry weight and wet weight of Goldfish using a subsample of 28 individuals.

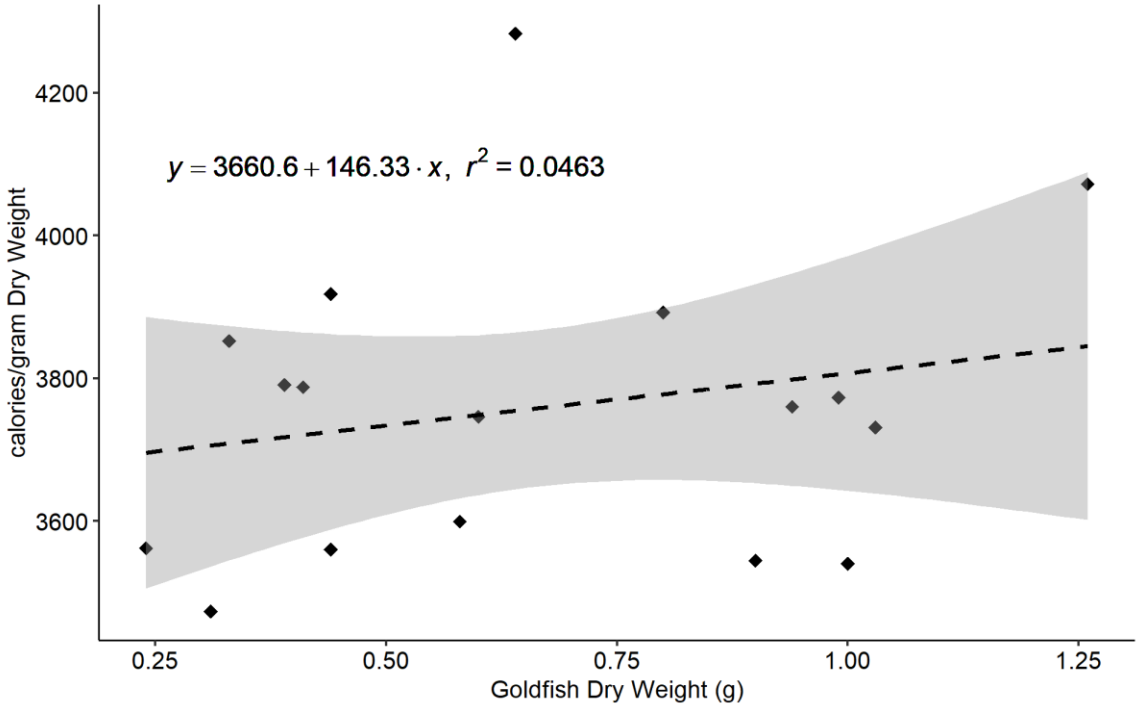


Figure 3-2. A simple linear regression showing no relationship between dry weight and calories/g dry weight of Goldfish using a subsample of 17 individuals.

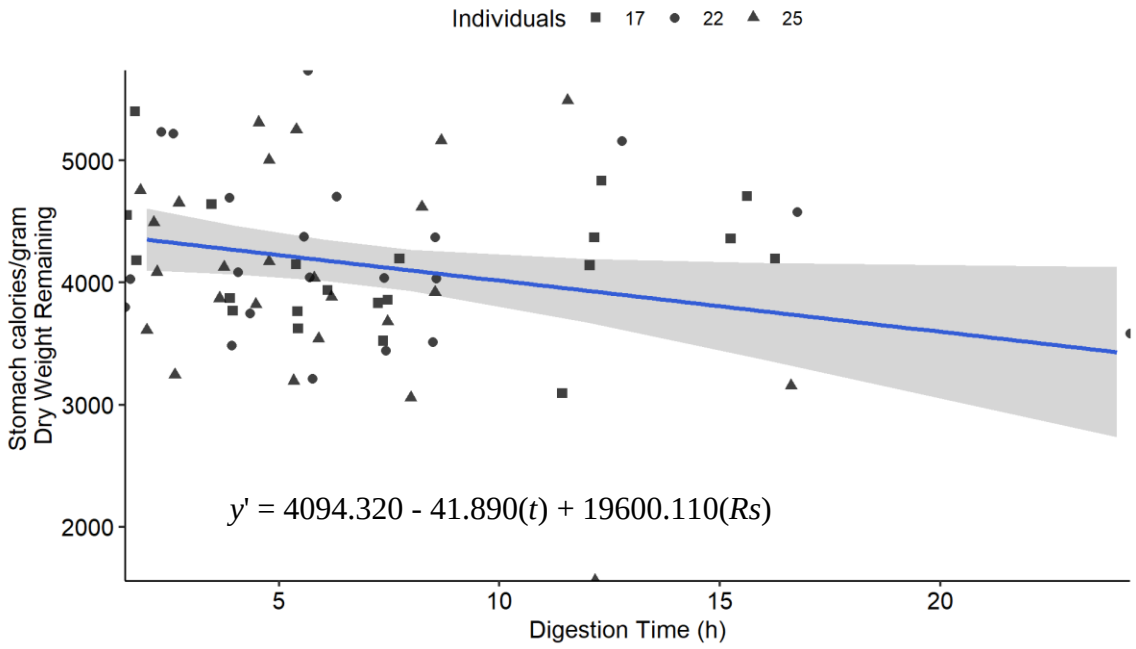


Figure 3-3. Generalized linear model of the calories/g dry weight remaining in stomach of juvenile Flathead Catfish (*y*') based on digestion time (*t*) and ration size (*Rs*). Individuals bombed stomach contents are shown for each temperature treatment (17º C = squares, 22º C = circles, and 25º C= triangles). Ration size was held constant with the mean value 0.015 to predict the regression.

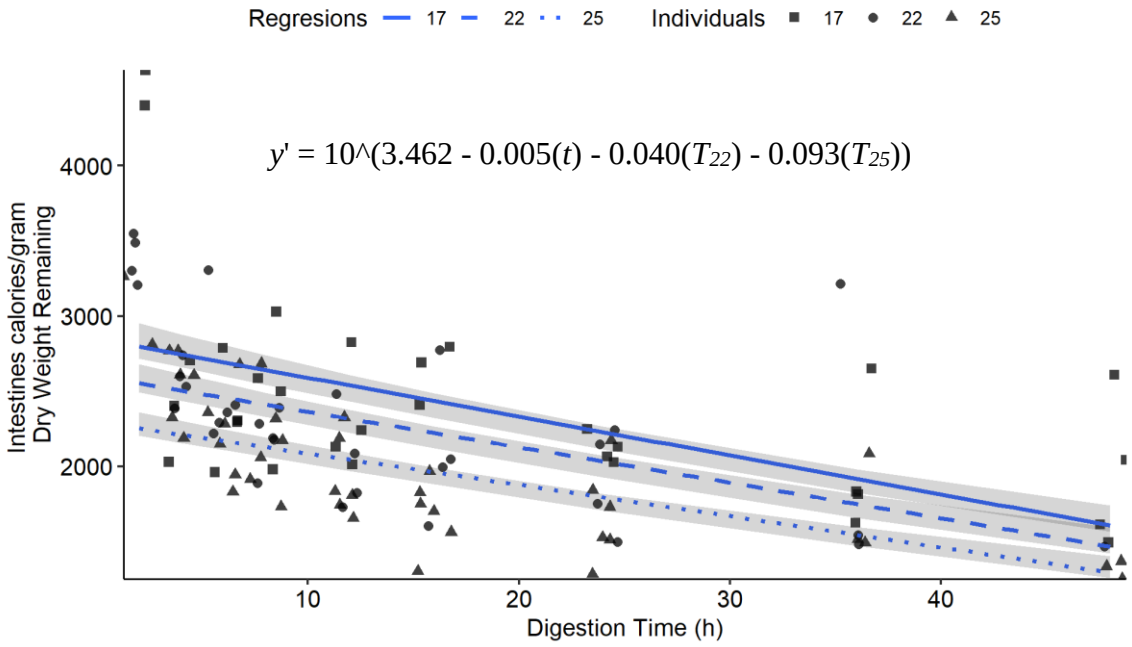


Figure 3-4. Generalized linear model of the back transformed calories/g dry weight remaining in intestines of juvenile Flathead Catfish (y') based on digestion time (t) and treatment temperature (17º C, solid line; 22º C, dashed line; 25º C, dotted line). T_{22} and T_{25} are binomial inputs (0 or 1) used to examine treatment temperature 22º C (T_{22} = 1 and T_{25} = 0) and 25º C (T_{22} = 0 and T_{25} = 1), 17º C is the reference group T_{22} = 0 and T_{25} = 0. Individuals bombed intestine contents are shown for each temperature treatment (17º C, squares; 22º C, circles; and 25º C, triangles).

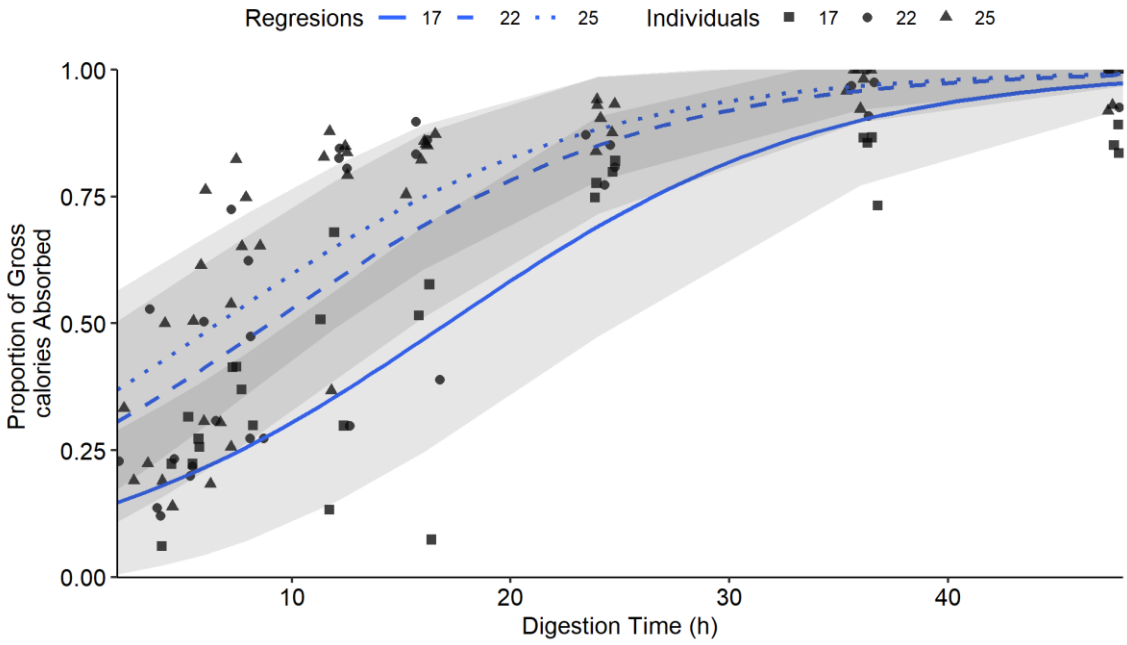


Figure 3-5. Binomial logistic regression for each treatment (17º C, solid line; 22º C, dashed line; and 25º C, dotted line) on the proportion of the gross calories absorbed by juvenile Flathead Catfish (n =126); based on all nine digestion times (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h) and a mean ration size of 0.015. Regression overlays the proportion of the gross calories absorbed by individuals (17º C, squares, n = 30; 22º C, circles, n = 35; and 25º C, triangles, n = 50). The proportion of gross calorie absorption was not different among treatments (α = 0.05).

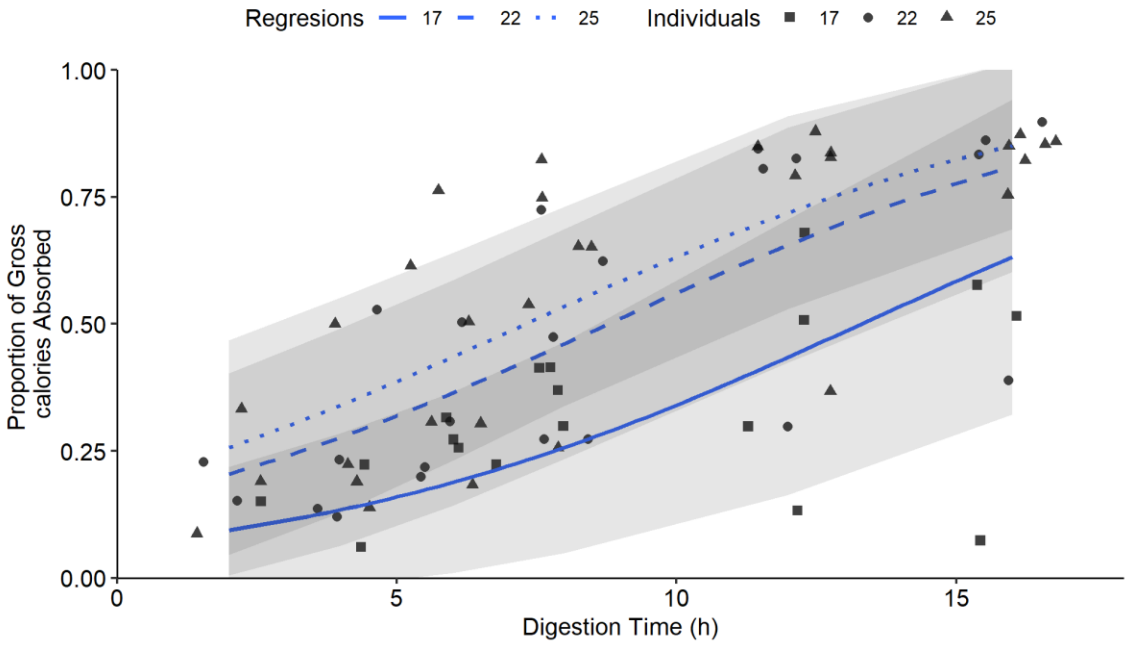


Figure 3-6. Binomial logistic regression for each treatment (17º C, solid line; 22º C, dashed line; and 25º C, dotted line) on the proportion of the gross calories absorbed by juvenile Flathead Catfish (n =126); based on restricted digestion times (2h, 4h, 6h, 8h, 12h, 16h) and a mean ration size of 0.015. Regression overlays the proportion of the gross calories absorbed by individuals (17º C, squares, n = 18; 22º C, circles, n = 23; and 25º C, triangles, n = 31). The proportion of gross calorie absorption was not different among treatments (α = 0.05)

GENERAL CONCLUSIONS

The aim of our study was to better understand the effect of temperature on the evacuation rates and absorption efficiencies of juvenile Flathead Catfish. We found temperature significantly effects the gastric evacuation rate of juvenile Flathead Catfish, but the rate of meal passage through the intestines was not different among our temperature treatments (17º C, 22º C, and 25º C). Overall, gastric evacuation time were found to differ by more than 5 hours between fish fed a single ration of food at 17º C and 25º C.

An increase in the gastric evacuation rate of juvenile Flathead Catfish suggests that consumption rates will increase as temperature increases. Our results found an increase in consumption as temperature increased; we observed 43% of Flathead Catfish consumed a Goldfish at 17º C, 58% of Flathead Catfish consumed a Goldfish at 22º C, and 81% of Flathead Catfish consumed a Goldfish at 25º C. There was a difference in consumption rates between 17º C and 25º C as well as 22º C and 25º C Flathead Catfish. We beleive that consumption increased to meet the growing metabolic demands of Flathead Catfish at higher temperatures. Despite the differences observed in consumption rates and gastric evacuation rates, the rate at which meals passed through the intestines were not different among treatments.

The calories/g DW of food remaining within the stomach were not different between treatment temperatures, however time of digestion was a significant predictor.

Digestion is suggested to have occurred in layers, and easy to digest content high in caloric value was passed from the stomach into the intestines resulting in a gradual decrease in observed calories/g DW in the stomach. In contrast, we found that the calories/g DW remaining within the intestines were different between 17º C and 25º C as well as 22º C and 25º C treatments. Likely, the largest amount of intestinal absorption occurred soon after the meal had passed into the intestines and was in a liquefied state.

The absorption of gross calories was not different between treatments. This suggests that juvenile Flathead Catfish are capable of absorbing a similar maximum amount of calories from the food source regardless of temperature. This finding is important in that we predicted potential energy to be lost at warmer temperatures due to faster evacuation rates. Absorption efficiencies may increase for fish held in warmer temperatures to compensate for faster passage of the meal. Our experiment could not distinguish between energy lost due to egestion and excretion or that lost to metabolic demands, storage, or production (growth). Future work should focus on energy partitioned to various components of the bioenergetics models (e.g., specific dynamic action, activity, waste, etc.). This would provide managers with a more holistic idea of net energy devoted towards growth of Flathead Catfish.

We also observed differences between the number of individuals that fed at night (n=98) versus those that fed during the day (n=28), suggesting that feeding behavior was influenced by photoperiod within the laboratory. Laboratory water was clean and clear and future studies should examine the effects that lighting has on turbid water, which would be more indicative of the natural waters where Flathead Catfish are found.

However, our behavioral results coupled with gastric evacuation rates provide managers with a general idea of when most feeding is occurring and roughly how long stomach contents are present at various temperatures. Collectively, our work can help inform field studies of Flathead Catfish, as these findings provide managers with a guideline of when they should sample stomachs of wild fish (both seasonally and diurnally) and when to expect higher occurrences of empty or full stomachs.

Our experiment lays the groundwork for future studies to continue research and focused manipulations on the effect of temperature on the behavior and physiology of juvenile Flathead Catfish. In our examination of gastric evacuation rates and absorption efficiencies, we accounted for a single ration of a single food source. Wild populations of juvenile Flathead Catfish are inclined to consume rations opportunistically, which will vary in size and prey type. Future work should focus on a variety of food sources and quantities. Additionally, the experimental temperatures we choose to examine were ecologically narrow for the range of temperatures that Flathead Catfish can be found. Examining a wider range of temperatures could provide insight into which temperatures are optimal for consumption, metabolic demand, and net absorption of calories.

Overall, our work was unique and innovative for Flathead Catfish, as they have rarely been reared in a laboratory setting. Our work allowed us to empirically quantify changes in behavior and physiology when isolating temperature, the “abiotic master variable.” Our results further our understanding of the species, and improve our understanding of gastrointestinal physiology of fish in general.

APPENDICES

Appendix 1. A note on rearing Flathead Catfish *Plyodictis olivaris* in a recirculating aquatic system

Introduction

The earliest known reference of "aquahusbandry" occurred in China and dates back to 475 BC when a merchant referenced how to create a viable business culturing carp (Nash 2011). However, the first commercial aquaculture industries in the United States did not emerge until 1960's, with the rearing and production of Channel Catfish, *Ictalurus punctatus* (Welborn 1983). Since the 1960's, advances in technology along with the creation of a profitable market have propelled the global growth of fish production in aquaculture to an estimated 52.5 million tonnes worth $98.5 billion in 2008 (Bostock et al. 2010). Production of catfishes dominates the aquaculture industry in the U.S. in terms of weight and makes up 40% of the total estimated value of the aquaculture industry (USDA 1995).

Fast growth rates coupled with pleasant meat palatability showed promise for Flathead Catfish *Plyodictis olivaris* as a commercial species in aquaculture (Swingle 1954). Flathead Catfish are noted as one of the fastest growing freshwater fish in North America based on length-at-age data (Jackson 1999). Most individuals reach maturity by age three or a total length around 40 cm. Their maximum lengths and weights can be over 150 cm and more than 45 kg (Hrabik et al. 2015). However, major drawbacks deter from the production of Flathead Catfish in aquaculture.

Descriptions of Flathead Catfish diet do not suggest that feeding Flathead Catfish in captivity would be a challenge. For example, Flathead Catfish have been classified as opportunistic feeders (Pine et al. 2005; Turner 2017). Diet varies based on life history stage and food availability in the environment, with juvenile Flathead Catfish eating primarily insect larvae before becoming more aggressive piscivores as adults (Minckley and Deacon 1959; Roell and Orth 1993; Hogberg and Pegg 2011). However, feeding Flathead Catfish has presented one of the largest obstacles in maintaining fish health in an aquaculture setting (Snow 1959; Moen 1964). Additionally, Flathead Catfish have been considered unsuitable for commercial culture because of cannibalistic tendencies, unlike easier to culture species such as Channel Catfish and Blue Catfish *Ictalurus furcatus* (Tucker and Robinson 1990). Propagation of Flathead Catfish in captivity has also had limited success (Johnson 1950, Snow 1959).

The ability to rear and cultivate fish in aquaculture has benefits beyond fish production for consumption or sport fish stocking. As technology and literature surrounding aquaculture grows, so do the opportunities to rear and study species beyond those that have traditionally been reared in production facilities. For example, opportunities are becoming more available to propagate and stock endangered fish species (e.g., spawning the rare pygmy madtom, *Noturus stanauli* 2000). Recirculating aquaculture systems give scientists and managers versatility in the ability to control for abiotic and biotic factors which is useful in experimental design. Recirculating aquatic systems also provide a means of intensive fish production while limiting environmental

impacts by reusing water and improving waste management and nutrient cycling (Martins et al. 2010).

Understanding the behavior and physiology of a species should be the first step in the design and development of a recirculating aquatic system. Considerations for rearing tank size are dependent on species density limitations. For example, Espmark et al. (2016) found that larger tank size lead to increased feed ingestion and growth of Atlantic salmon *Salmo salar*. Similarly, the shape of tanks (e.g., round vs. rectangular) is dependent on swimming characteristics of the species and influence flow rates within tanks.

Life-history provides insight on the equipment necessary to support sufficient water quality for the species. For example, understanding the thermal limits of a species will impact appropriate water heater or chiller configurations. Sensitivity to ammonia, nitrate, and nitrite will dictate fish densities and the appropriate size of the bio-filtration systems. Selecting appropriate equipment helps mitigate stress of individuals within a recirculating aquatic system.

Stress can alter both the behavior and physiology of a species, which can lead to false conclusions to research questions. Furthermore, stress can alter growth rates (Pickering 1993) and fish condition, which negatively influence fish tissue (i.e., flesh) production important within aquaculture settings used for profit as well as studies interested in examining growth.

Laboratory studies conducted within a recirculating aquatic system allow scientists to quantify species-specific physiological processes and ask targeted questions

that evaluate the responses of fishes to various environmental conditions, largely due to the amount of control exerted over a recirculating aquatic system. While research has provided a great deal of information regarding rearing of certain species of fish, data are sorely lacking for others. Flathead Catfish are one such species for which little documentation exists on rearing and husbandry. A lack of documentation on past aquaculture practices of Flathead Catfish hinders future studies within laboratory settings.

Our objective was to address the knowledge gap that exists in rearing Flathead Catfish in a recirculating aquatic system. Developing an efficient rearing system will allow us to effectively evaluate how environmental conditions may alter the behavior and physiology of Flathead Catfish. Following the general guidelines of rearing fish in a laboratory as reviewed by DeTolla et al. (1995), we designed, developed, and operated three recirculating aquatic systems capable of rearing Flathead Catfish. Due to the lack of documentation, we outline the research and development of a recirculating aquatic system capable of rearing Flathead Catfish.

System Design

We retrofitted a wet laboratory with three independent recirculating aquatic systems with a collective capacity of 16,500 L of water. City water was filtered through two 312 L Vantage PTC Carbon Filters (Evoqua, Rockford, Illinois, U.S.A.) before it was passed through a Pentek Big Blue Microfilter (Pentair, Minneapolis, Minnesota, U.S.A.) and entered into a 3785 L reservoir. The 3785 L reservoir of filtered water was then either heated or cooled depending on which system the water was diverted to during

water changes. From the reservoir, water was pumped into one of the three recirculating aquatic system's sump tanks (1892 L). Each recirculating aquatic system was independent of the others, allowing us to control the temperature within each system. Each system was gravity fed by the sump. For each system, water entered one of four mesocosms (diameter = 1.07 m, height = 1.07 m) via a spray bar. A standpipe within each mesocosm was set to hold roughly 900 L of water. A variable speed FloPro pump (Jandy, Carlsbad, California, U.S.A.) was used to pull water from the rearing tanks into an Arias 8000 fiberglass aquaculture sand filter (Pentair, Minneapolis, Minnesota, U.S.A.) where large particles were filtered out. Water then passed through a Delta Star in-line water chiller (Aqualogic, San Diego, California, U.S.A.) controlled by an digital temperature controller (Aqualogic, San Diego, California, U.S.A.). Water then went through a Sweetwater Low-Space Bioreactor (Pentair, Minneapolis, Minnesota, U.S.A.). Beneficial bacteria within the bioreactor reduced ammonia and nitrite levels before the water was gravity fed through a Smart UV High-output Sterilizer (Pentair, Minneapolis, Minnesota, U.S.A.). The UV light was used to help combat parasite infestations within the system. From the UV light, water was gravity fed back into the systems' sump tank before it was gravity fed into one of the four mesocosm tanks to continue along this cycle (See Chapter 2, Figure 2-1). On average, our systems cycled once every 1.7 hours, which equated to an average flow of 45.4 L per minute.

Prior to introduction of Flathead Catfish within the laboratory, we cycled our systems for 3 weeks to ensure functionality. During this time, Bluegill *Lepomis*

macrochirus were introduced to facilitate the growth of a colony of bacteria within the bioreactor to initiate nitrogen cycling.

Source, collection, and transport

One area of concern for introducing a species into a laboratory space is the origin source. Unlike well-studied organisms with established aquaculture practices already in place, researchers will need to rely on a native source of wild caught fish. In addition to a source of fish, researchers will have to consider optimal methods of capture (time of year, type of gear), transportation (how far back to the laboratory, etc.), and permitting restrictions. It is critically important for researchers to minimize stressors during transport, including those imposed due to handling, crowding, and water quality conditions (Wynne and Wurts 2011). For example, Tacchi et al. (2015) examined one method of stress management techniques using salt to mitigate changes to the fish's skin during transport, which can be a simple and cost-effective method of reducing energetic burden during transport. Finding methods that cause the least amount of stress are necessary to increase the probability of survival throughout the transport phase of introducing wild fish into a research laboratory setting.

Initial collection of Flathead Catfish occurred in the summer of 2018 from the Missouri River near Nebraska City, Nebraska, U.S.A. We collected Flathead Catfish using a 15 Hz, 3-4 amp pulsed DC electric field produced by a generator (Kohler, Kohler, Wisconsin, U.S.A.) and 5.0 GPP pulsator (Smith-Root, Vancouver, Washington, U.S.A.) mounted on a boat. We targeted Flathead Catfish ranging in size from 200 mm to 400

mm in total length to reduce variability of life-history differences. Flathead Catfish were held in a live well on the boat until collection concluded. We transported Flathead Catfish back to the laboratory using aerated coolers (45 L). We conducted two sampling trips until roughly 200 Flathead Catfish were collected.

Acclimation to laboratory

We randomly distributed Flathead Catfish between the three recirculating aquatic systems, sorting by size within each system to reduce the possibility of cannibalism. Each system contained roughly 70 individuals needed for experimentation (Chapter 2 of this thesis). We achieved temperature acclimation via a 1° C increase or decrease per day until the desired temperature was reached (17º C, 22º C, or 25º C).

Diet

We assessed different sources of food suitable for juvenile Flathead Catfish to maintain healthy condition within the laboratory. Past research has shown varied success in attempts to getting Flathead Catfish fry to consume pelleted feed (Moen 1964). We attempted to feed our juvenile Flathead Catfish a floating commercial grade feed typically consumed by Channel Catfish (Rangen Inc., Buhl, Idaho, U.S.A.). However, Flathead Catfish showed no interest in this feed type and we did not observe feeding. We never left pelleted feed in our rearing tanks longer than 10 minutes. Due to our tank design, floating feed was occasionally pulled down the standpipes and into our sand

filters which altered water quality (e.g., spiking nitrate levels) and required immediate water changes.

We next attempted a commercial sinking feed (Rangen, Buhl, Idaho, U.S.A.) commonly consumed by Channel Catfish. We assumed the pelleted feed lying on the bottom of the tank was more likely to be consumed by a benthic-oriented species. Juvenile Flathead Catfish remained sedentary and showed no interest in this food source. Excess food had to be pulled quickly to reduce leaching of nutrients into the water and affecting overall water quality. We found it evident that wild Flathead Catfish were unlikely to consume an artificial commercial pelleted diet, and thus terminated the introduction of pelleted food.

Alternatively, we evaluated earthworms *Lumbricus Terrestris*, frozen brine shrimp *Artemia*, and freeze-dried bloodworms *Glycera* as a food source. We observed minimal feeding of earthworms at warmer temperatures (25º C) and no feeding at the cooler temperatures (17º C and 22º C). Juvenile Flathead Catfish did not consume brine shrimp. Earthworms and the brine shrimp both affected visibility of the water, although overall water quality did not seem to be affected (e.g., no harmful spikes in nitrate). Flathead Catfish did not consume freeze-dried bloodworms as this food source floated on the surface of the water and eventually was dissipated down the standpipe.

The most successful food source provided was live fish. We fed Flathead Catfish live Fathead Minnows *Pimephales promelas* at a rate of 2% of the catfish bodyweight per fish per day. Fathead minnows were sourced from a commercial vendor (Rainbow Bait, Ruthton, Minnesota, U.S.A.) and were quarantined in the laboratory for at least two

weeks prior to being fed to the Flathead Catfish. Fathead Minnows were replenished each morning in the laboratory and Flathead Catfish consumed their meals voluntarily. Anecdotal evidence showed that Flathead Catfish were primarily consuming their meals during nighttime hours (12 hour light, 12 hour dark photoperiod) in the laboratory and remained sedentary in the tanks during the daylight hours.

High feeding rates of Flathead Catfish required two additional tanks (e.g., one quarantine tank and one holding tank) to be set up within our laboratory for maintaining a population of healthy Fathead Minnows. Due to the high density of Fathead Minnows, we found it most efficient and cost effective to maintain flow through systems. Fathead Minnows were fed crushed pelleted feed, and we replenished their population by the commercial vendor every two weeks.

Parasites

Ichthyophthirius multifiliis (Ich) and *Trichodina* (Trich) caused a high mortality rate of Flathead Catfish in our initial laboratory setup. Unlike Channel Catfish that show symptoms of white spots on their skin, Ich on Flathead Catfish was not visible to the naked eye. Skin scrapes and gill samples were examined under a microscope to detect the protozoa. Due to the life cycle of Ich, recirculating aquatic system #3 (Treatment temperature 25° C) had the most rapid decline in fish health and subsequent mortality of our Flathead Catfish. Recirculating aquatic system #2 (Treatment temperature 22° C) experienced losses at the same time as recirculating aquatic system 3 but over a more prolonged time period. Ultimately, recirculating aquatic system #2 experienced a

complete mortality event. Recirculating aquatic system #1 (Treatment temperature 17° C) also experienced losses, however, we were able to slow and eventually eradicate the protozoa within the system prior to complete mortality.

We found salt treatments were unsuccessful in combating disease within our recirculating aquatic systems. Treatments of salt were difficult to conduct within the recirculating aquatic systems, and salt dips were highly stressful and ineffective. Therefore, we explored alternative treatments for parasites.

We evaluated treatments of formalin using similar methodologies previously found to be successful with Channel Catfish (e.g., Tieman and Goodwin 2001). Roughly half of the water in each system was drained to reduce the amount of formalin needed for treatments. We added formalin to each system to achieve 125 ppm. Ich is only susceptible to formalin during a "free-swimming" portion of its life cycle. Once the parasite attaches to the host fish and creates cysts, they are no longer easily treated by formalin as the fish's mucus layer protects the Ich (Tieman and Goodwin 2001). Therefore, it was crucial to treat the system in its entirety and not just isolate the fish in a water treatment bath.

To treat a system, the system pump was active, allowing the treatment to recirculate throughout the system. We installed a by-pass valve on each systems' biofilter to ensure formalin did not harm beneficial colonies of bacteria within the bioreactors. Water was allowed to circulate for 30-45 minutes before we flushed and refilled to the system using water (within ±1° C of the system) from the treated reservoir. We conducted non-lethal skin scrapes daily on randomly selected individuals to assess

efficacy of the formalin treatments. Different water temperatures resulted in different lengths of treatment needed to eradicate the protozoa infection. Water temperatures below 25° C required an additional week of treatment due to the life cycle of the parasites. Daily treatments mitigated and eventually ceased losses due to ectoparasites.

Occasionally, during the acclimation phase to the laboratory, Flathead Catfish would become infected with the bacterial disease *Flavobacterium columnare*, also known as columnaris disease. Columnaris was a secondary infection that likely resulted from poor feeding or poor water quality. Symptoms included skin lesions and fin erosion. At first sign of the infection, API Melafix (Mars Fishcare North America Inc., Chalfont, Pennsylvania, U.S.A.) was introduced to the system, which contains 1% Cajeput (tea tree oil) to treat infected fish. Treatments lasted for 7 days and resulted in the healing of the bacterial infection.

The possibility of introducing disease within a system was higher when introducing other live fish as a food source. We designed a quarantine procedure for all incoming Fathead Minnows prior to introduction to Flathead Catfish tanks. Daily hour-long treatments of formalin were conducted on the newest delivery of Fathead Minnows for a two-week period. Once treated, Fathead Minnows were moved to a holding system that we pulled daily rations for Flathead Catfish. This constant movement allowed for seamless transition of incoming fish to quarantine tanks, from quarantine tanks to holding tanks, and finally into the recirculating aquatic systems. We found this method most suitable as there were no outbreaks of parasites or diseases due to introduced food sources throughout our holding and rearing period. Costs for disease management were

minimal and only conducted within the acclimation phase of introducing fish into our laboratory.

Stress Management

Paramount to the health of juvenile Flathead Catfish is managing stress levels within the laboratory. Chronic stress can lead to maladaptation, meaning regulatory mechanisms cannot compensate the effects of a stressor, ultimately resulting in death (Balasch and Tort 2019). Stress is often additive for fish species. For example, stress can affect fish and cause primary responses (e.g., hormone changes), secondary responses (e.g., metabolic changes), and tertiary responses (e.g., changes in whole animal health; Barton 2002). We used shade cloth to reduce lighting within rearing tanks and 3-inch PVC pipes placed freely on the bottom of tanks to provide structure for fish to hide in. Additionally, we monitored behavior and health of individuals to ensure stress did not exceed to a level at which the fish could not survive.

We primarily used low feeding rates and poor skin health as indicators of stress in juvenile Flathead Catfish. When stress was indicated through observation of these metrics, we conducted daily water quality tests and close monitoring of individuals until healthy behavior resumed. Disregarding the initial issue in selecting an appropriate diet within the laboratory, we primarily noted high levels of stress during transport and acclimation to the laboratory as well as during formalin treatments. Following this brief period (approximately 3 weeks), we did not observe any indication of stress for the

remainder of our experiment. However, we continued to take caution when removing shade cloth covers and siphoning systems not to disturb individuals.

Refinement and re-introduction

Due to the high mortality rates associated disease, we collected another cohort of Flathead Catfish from the Missouri River (Blair, Nebraska, U.S.A.) in the fall of 2018 (n=175). We selected for juveniles Flathead Catfish, total length ranged from 174 mm to 405 mm. We randomly distributed Flathead Catfish between tanks and acclimated them to experimental temperatures over a period of three weeks. Upon arrival to the laboratory we noticed high levels of feces and regurgitation from previous meals. This was noted as a stressful event and we monitored water quality and feeding closely over the acclimation period. We implemented additional siphoning for waste materials to help alleviate poor water conditions. Daily rations of Fathead Minnows were introduced the same evening Flathead Catfish arrived to the laboratory.

We conducted daily formalin treatments at 125 ppm and until no sign of ectoparasites were visible in random skin scrapes. Entire systems were treated with Melafix at the first sign of columnaris on any individual. Flathead Catfish were actively using PVC fittings as structure within the tanks. Once the initial acclimation period ended, medicinal treatments for both parasites and bacteria were unnecessary for the duration of the gastric evacuation rate experiment (Chapter 2 of this thesis). The successful acclimation, rearing, and maintaining condition for our sample spanned from

September 24, 2018 to February 4, 2019. We did not observe any natural mortality of the 210 juvenile Flathead Catfish over the 133 days.

Conclusions

Collection of wild fish from their native habitat, followed by transportation and introduction into artificial conditions are extremely stressful events for fishes. One or any combination of these stressful events can cause mortality if an individual is unable to compensate physiological regulation. While previous work has shown that Flathead Catfish are capable of recovering from electroshocking (Morris 2018) and transportation, we know little in regards to their ability to cope with stress within a recirculating aquatic system. We found it was possible to mitigate stress to ensure survival once juvenile Flathead Catfish were introduced into the laboratory by focusing on three major components. These included providing a live food source (e.g., Fathead Minnows fed at a rate of 2% per day) to maintain high daily feed rates, eradicating ectoparasites upon introduction to the laboratory, and maintaining water quality. Additionally, we found it necessary to provide this species with a dark photoperiod as a behavioral cue to encourage feeding. Shade cloth over tanks along with structure within tanks reduced stress and ensured a healthy population of juvenile Flathead Catfish. Daily siphoning of large waste particles, weekly water changes of 50% of the system, and bi-weekly backwash of our sand filters promoted healthy water quality through time. Using these protocols, we were capable of rearing healthy juvenile Flathead Catfish for 134 days without any natural mortality.

Aquaculture systems come in a variety of configurations suitable for many species, including non-native species. Negative impacts of certain aquaculture techniques have been reviewed. Diana (2009) provided an overview of threats to biodiversity due to aquaculture which include: escapement and genetic alterations of wild stocks, effluent's negative effect on water quality, inefficient resource use, and disease transfer from captive to wild stocks. Non-native fish can pose a significant threat to native species when reared in traditional aquaculture practices (e.g., escapement from earthen ponds due to flooding). Recirculating aquatic systems provide producers, managers, and researchers an alternative method of rearing or studying fish in a setting that mitigates threats of escapement and interaction with wild populations. Flathead Catfish are one such species that pose a threat to biodiversity in their non-native ranges (Hilling et al. 2019). They are a voracious apex predator that can quickly impact native fish populations where they are introduced (Pine et al. 2007). Recirculating aquatic systems provide the means to conduct research in a biologically safe manner in areas where escapement is a concern.

As researchers, we have the ethical responsibility to ensure animal welfare. This is especially true when taking wild animals into captivity. The main goal of animal welfare within a recirculating aquatic system is to maximize productivity and minimize stress and mortality (Espinal and Matulic 2019). Providing researchers with a basic scientific understanding of a recirculating aquatic system setup for species rarely cultured in a laboratory reduces time and effort in experimental design that could be focused on research questions. We believe highlighting a detailed procedure of setting up our

recirculating aquatic system along with the shortcomings we experienced will provide managers and researchers valuable insight into rearing Flathead Catfish.

Unpredictable events occur when introducing a species into artificial conditions. Allowing room for flexibility in a recirculating aquatic system's design is necessary. Badiola et al. (2012) found that over 50% of recirculating aquatic systems require alteration or rebuilding after fish are introduced due to poor system design, water quality issues, and mechanical failures. In setting up our recirculating aquatic systems, we altered our design plan multiple times during the building phase to accommodate the needs of our species. Versatility in system design (control valves) allowed us to make alterations to our systems (e.g., install UV lights and flow switches, increase head by raising biofilter height, etc.) while fish were living in the laboratory. Having the ability to adapt to various conditions that arise during rearing of fish within the laboratory is paramount to ensuring a healthy population. Behavioral cues of the individuals was a tell-tale way to determine if something was amiss on our systems. Proper system mechanics ultimately led to a heathy population of fish.

Due to the ability of raising a high density of individuals within a confined space, recirculating aquatic systems have been compared to feed lot style farming practices in terrestrial agriculture (Helfrich and Libey 2013). Aquaculture production facilities battle disease outbreak and increasing stress levels when trying to increase production yield (Mohapatra et al. 2012). Fish with life-history characteristics conducive to aquaculture tend to be easy to propagate, have fast growth rates, are cost-effective to feed, and can be maintained at high densities. Flathead Catfish fail to meet many of these characteristics,

which explains why previous literature within production facilities or a laboratory setting is sorely lacking for the species.

Our work provides evidence that is possible to rear juvenile Flathead Catfish within a recirculating aquatic system. These results lay the groundwork for laboratory studies examining key physiological and behavioral processes of this understudied apex predator.

LITTERATURE CITED

Badiola, M., D. Mendiola, and J. Bostock. 2012. Recirculating aquaculture systems (RAS) analysis: Main issues on management and future challenges. Aquacultural Engineering 51:26–35.

Balasch, J. C., and L. Tort. 2019. Netting the stress responses in fish. Frontiers in Endocrinology, 10:62.

Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and comparative biology, 42(3):517-525.

Bostock, J., B. McAndrew, R. Richards, K. Jauncey, T. Telfer, K. Lorenzen, D. Little, L. Ross, N. Handisyde, I. Gatward, and R. Corner. 2010. Aquaculture: global status and trends. Philosophical Transactions of the Royal Society B: Biological Sciences 365(1554):2897-2912.

Spawning the rare Pygmy Madtom, *Noturus stanauli*. 2000. Conservation Fisheries Inc. Newsletter #2, Knoxville, Tennessee.

DeTolla, L. J., S. Srinivas, B. R. Whitaker, C. Andrews, B. Hecker, A. S. Kane, and R. Reimschuessel. 1995. Guidelines for the care and use of fish in research. Institute for Laboratory Animal Research Journal 37(4): 159-173.

Diana, J. S. 2009. Aquaculture production and biodiversity conservation. BioScience 59(1):27-38.

Espinal, C. A., and D. Matulić. 2019. Recirculating aquaculture technologies. Pages 35–76. *In* S. Goddek, A. Joyce, B. Kotzen, and G. M. Burnell, editors. Aquaponics Food Production Systems. Springer International Publishing, New York, New York.

Espmark, Å.M., J. Kolarevic, T. Åsgård, and B.F. Terjesen. 2017. Tank size and fish management history matters in experimental design. Aquaculture Research 48(6):2876-2894.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293(5538): 2248-2251.

Helfrich L. A. and G. Libey. 2013. Fish farming in recirculating aquaculture systems (RAS). Available: http://fisheries.tamu.edu/files/2013/09/Fish-Farming-in-Recirculating-Aquaculture-Systems-RAS.pdf

Hilling, C. D., A. J. Bunch, J. A. Emmel, J. D. Schmitt, and D. J. Orth. 2019. Growth and mortality of invasive Flathead Catfish in the tidal James River, Virginia. Journal of Fish and Wildlife Management 10(2): 641–652.

Hrabik, R.A., S.C. Schainost, R.H. Stasiak, and E.J. Peters. 2015. The fishes of Nebraska. Mennonite Press, Newton, Kansas.

Jackson, D. C. 1999. Flathead Catfish: biology, fisheries, and management. Catfish 2000: proceedings of the international ictalurid symposium. American Fisheries Society, Symposium 24, Bethesda, Maryland.

Johnson, M. C. 1950. Some experiments on the propogation of the Flathead Catfish *Plyodictus olivaris* (Rafinesque) in ponds. Master's thesis. Alabama Polytechnic Institute, Auburn.

Minckley, W. L., and J. E. Deacon. 1959. Biology of the Flathead Catfish in Kansas. Transactions of the American Fisheries Society 88(4):344-355.

Moen, T. 1964. Feeding young Flathead Catfish. The Progressive Fish-Culturist 26(1):26–26.

Mohapatra, S., T. Chakraborty, V. Kumar, G. DeBoeck, and K. N. Mohanta. 2013. Aquaculture and stress management: a review of probiotic intervention: stress management by probiotics administration. Journal of Animal Physiology and Animal Nutrition 97(3):405–430.

Morris, W. Z. 2018. Determining electrofishing response thresholds of Smallmouth Bass *Micropterus dolomieu*, Flathead Catfish *Pylodictus olivaris*, and Blue Catfish *Ictalurus furcatus*. Master's thesis. University of Missouri, Columbia.

Nash, C. E. 2011. The history of aquaculture. Wiley-Blackwell, Ames, Iowa.

Omeji, S., J. O. Apochi, and K. A. Egwumah. 2017. Stress concept in transportation of live fishes – a review. Journal of Research in Forestry, Wildlife, and Environment 9(2):57-64.

Pickering, A. D. 1993. Growth and stress in fish production. Aquaculture 111:51-63.

Pine, W. E., III. T. J., Kwak, and J. A. Rice. 2007. Modeling management scenarios and the effects of an introduced apex predator on a coastal riverine fish community. Transactions of the American Fisheries Society 136(1):105–120.

Pine, W. E., III. T. J. Kwak, D. S. Waters, and J. A. Rice. 2005. Diet selectivity of introduced Flathead Catfish in coastal rivers. Transactions of the American Fisheries Society 134(4):901–909.

Roell, M. J., and D. J. Orth. 1993. Trophic basis of production of stream-dwelling Smallmouth Bass, Rock Bass, and Flathead Catfish in relation to invertebrate bait harvest. Transactions of the American Fisheries Society 122(1):46–62.

Snow, J. R. 1959. Notes on the propagation of the Flathead Catfish, *Pilodictis Olivaris (*Rafinesque). The Progressive Fish-Culturist 21(2):75–80.

Swingle, H. S. 1954. Fish populations in Alabama rivers and impoundments. Transactions of the American Fisheries Society 83(1):47-57.

Tacchi, L., L. Lowrey, R. Musharrafieh, K. Crossey, E. T. Larragoite, and I. Salinas. 2015. Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of Rainbow Trout *Oncorhynchus mykiss*. Aquaculture 435:120-127.

Tieman, D. M., and A. E. Goodwin. 2001. Treatments for ich infestations in channel catfish evaluated under static and flow-through water conditions. North American Journal of Aquaculture, 63(4):293-299.

Troell, M., R. L. Naylor, M. Metian, M. Beveridge, P. H. Tyedmers, C. Folke, K. J. Arrow, S. Barrett, A. S. Crépin, P. R. Ehrlich, Å Gren, N. Kautsky, S. A. Levin, K. Nyborg, H. Österblom, S. Polasky, M. Scheffer, B. H. Walker, T. Xepapadeas, and A. de Zeeuw. 2014. Does aquaculture add resilience to the global food system? Proceedings of the National Academy of Sciences 111(37):13257-13263.

Tucker, C. C., and, E. H. Robinson. 1990. Channel Catfish farming handbook. Springer, New York.

Turner, D. R. 2017. Trophic dynamics of Flathead Catfish in the Missouri River bordering Nebraska. Master's thesis. University of Nebraska, Lincoln.

USDA (U.S. Department of Agriculture). 1995. Overview of aquaculture in the United States. Available: https://www.aphis.usda.gov/animal_health/nahms/aquaculture/downloads/AquacultureOverview95.pdf

Wynne, F. S., and W. A. Wurts. 2011. Transportation of warmwater fish: equipment and guidelines. Southern Regional Aquaculture Center (SRAC) Publication 390. Available: http://agrilife.org/fisheries2/files/2013/09/SRAC-Publication-No.-390-Transportation-of-Warmwater-Fish-Equipment-and-Guidelines.pdf

Appendix 2. Summary information tables for Chapter 2.

Table A2-1: Summary information for Flathead Catfish dissected in system 1, treatment temperature of 17º C. Mean and standard deviation (SD) of Flathead Catfish total length (mm), Flathead Catfish weight (g), ration weight (g), ration size (meal size as a percent of Flathead Catfish weight), acclimation (º C), consumption (º C), and dissection temperatures (º C). Male, female and unknown sex (marked if we could not differentiate male or female gonads) are provided as a count.

Dissection Time Interval	N	Mean ± (SD) Flathead Catfish Total Length, mm	Mean ± (SD) Flathead Catfish Weight, g	Mean ± (SD) Ration Weight, g	Mean ± (SD) Ration Size	Mean ± (SD) Acclimation Temperature, º C	Mean ± (SD) Consumption Temperature, º C	Mean ± (SD) Dissection Temperature, º C	Male	Female	Unknown
2	3	282.33 (24.19)	206.53 (34.10)	3.47 (1.60)	1.69% (0.89)	16.97 (0.74)	16.63 (0.06)	16.63 (0.06)	2	1	0
4	3	306.67 (37.29)	257.63 (69.97)	3.86 (1.53)	1.54% (0.53)	16.63 (0.32)	16.73 (0.23)	16.73 (0.23)	1	2	0
6	4	303.25 (35.04)	236.32 (66.47)	2.36 (0.96)	0.98% (0.24)	16.43 (0.26)	16.95 (0.69)	16.95 (0.69)	1	2	1
8	4	292.00 (29.70)	217.07 (46.72)	2.84 (1.64)	1.23% (0.53)	17.23 (0.49)	16.93 (0.81)	16.93 (0.81)	0	3	1
12	4	310.25 (39.45)	282.77 (93.68)	2.79 (0.73)	1.09% (0.48)	16.80 (0.29)	16.48 (0.17)	16.77 (0.42)	1	2	1
16	3	244.00 (63.84)	159.00 (110.75)	2.57 (0.54)	2.80% (2.75)	16.77 (0.38)	16.90 (0.40)	17.20 (0.53)	2	1	0
24	4	343.75 (32.38)	370.88 (119.01)	3.05 (0.48)	0.90% (0.33)	16.95 (0.37)	16.77 (0.34)	17.18 (0.59)	2	2	0
36	4	302.50 (49.03)	251.75 (130.57)	3.66 (1.34)	1.76% (0.85)	17.15 (0.51)	17.25 (0.61)	16.93 (0.64)	1	3	0
48	4	328.75 (69.70)	311.12 (149.45)	2.77 (1.06)	1.11% (0.74)	17.23 (0.42)	16.57 (0.52)	17.18 (0.39)	1	3	0

Table A2-2: Summary information for Flathead Catfish dissected in system 2, treatment temperature of 22º C. Mean and standard deviation (SD) of Flathead Catfish total length (mm), Flathead Catfish weight (g), ration weight (g), ration size (meal size as a percent of Flathead Catfish weight), acclimation (º C), consumption (º C), and dissection temperatures (º C). Male, female and unknown sex (marked if we could not differentiate male or female gonads) are provided as a count.

Dissection Time Interval	N	Mean ± (SD) Flathead Catfish Total Length, mm	Mean ± (SD) Flathead Catfish Weight, g	Mean ± (SD) Ration Weight, g	Mean ± (SD) Ration Size	Mean ± (SD) Acclimation Temperature, ºC	Mean ± (SD) Consumption Temperature, ºC	Mean ± (SD) Dissection Temperature, ºC	Male	Female	Unknown
2	4	310.75 (78.77)	312.75 (210.67)	3.25 (1.36)	1.37% (0.79)	22.05 (0.33)	21.82 (0.30)	21.82 (0.30)	1	2	1
4	4	284.25 (69.76)	243.10 (190.57)	2.67 (0.81)	1.56% (1.07)	22.30 (0.58)	22.00 (0.50)	22.00 (0.52)	2	1	1
6	5	260.60 (34.59)	167.54 (73.60)	2.84 (1.24)	2.26% (2.03)	21.88 (0.60)	21.80 (0.68)	21.80 (0.68)	3	1	1
8	5	268.40 (69.89)	202.08 (145.19)	2.81 (0.75)	1.95% (1.15)	22.12 (0.37)	22.48 (0.58)	22.48 (0.58)	2	2	1
12	4	312.50 (42.91)	282.73 (121.98)	2.39 (0.75)	0.98% (0.57)	21.90 (1.04)	22.25 (0.80)	22.52 (0.21)	1	2	1
16	4	258.50 (59.32)	179.57 (121.12)	2.76 (1.00)	2.06% (1.42)	21.65 (0.33)	22.05 (1.17)	22.25 (0.52)	2	2	0
24	4	302.75 (59.96)	265.90 (143.00)	3.50 (1.80)	2.13% (2.12)	22.62 (0.60)	22.15 (0.54)	21.98 (0.67)	2	2	0
36	4	278.75 (61.30)	237.88 (173.68)	3.02 (0.81)	1.63% (0.68)	21.95 (0.82)	22.18 (0.61)	21.90 (0.50)	1	2	1
48	4	318.75 (56.77)	296.40 (132.28)	2.93 (1.65)	1.11% (0.56)	21.65 (0.58)	22.12 (0.29)	22.30 (0.53)	4	0	0

Table A2-3: Summary information for Flathead Catfish dissected in system 3, treatment temperature of 25º C. Mean and standard deviation (SD) of Flathead Catfish total length (mm), Flathead Catfish weight (g), ration weight (g), ration size (meal size as a percent of Flathead Catfish weight), acclimation (º C), consumption (º C), and dissection temperatures (º C). Male, female and unknown sex (marked if we could not differentiate male or female gonads) are provided as a count.

Dissection Time Interval	N	Mean ± (SD) Flathead Catfish Total Length, mm	Mean ± (SD) Flathead Catfish Weight, g	Mean ± (SD) Ration Weight, g	Mean ± (SD) Ration Size	Mean ± (SD) Acclimation Temperature, ° C	Mean ± (SD) Consumption Temperature, ° C	Mean ± (SD) Dissection Temperature, ° C	Male	Female	Unknown
2	6	295.50 (34.25)	231.88 (84.06)	3.22 (0.61)	1.55% (0.58)	25.02 (0.41)	24.87 (0.44)	24.83 (0.48)	3	2	1
4	6	321.83 (54.11)	304.10 (153.10)	4.26 (1.53)	1.60% (0.82)	24.75 (0.34)	24.62 (0.24)	24.67 (0.22)	3	2	1
6	6	311.33 (48.27)	267.17 (114.74)	2.45 (0.55)	1.19% (0.83)	25.12 (0.34)	25.02 (0.31)	25.02 (0.31)	4	2	0
8	6	287.17 (36.93)	211.90 (77.86)	2.60 (1.17)	1.34% (0.65)	25.07 (0.36)	24.82 (0.31)	24.82 (0.31)	3	2	1
12	6	311.17 (64.73)	303.92 (160.09)	2.74 (0.99)	1.60% (1.76)	24.97 (0.44)	24.77 (0.22)	24.87 (0.34)	3	1	2
16	6	328.00 (55.73)	345.83 (211.41)	2.84 (1.23)	1.06% (0.62)	24.78 (0.31)	24.78 (0.23)	24.80 (0.31)	1	4	1
24	6	305.50 (45.15)	253.85 (110.47)	3.25 (0.85)	1.39% (0.35)	24.82 (0.38)	24.87 (0.23)	24.83 (0.34)	2	2	2
36	6	285.50 (28.62)	196.93 (69.53)	2.80 (0.76)	1.62% (0.85)	24.83 (0.31)	24.85 (0.26)	25.10 (0.39)	4	2	0
48	7	305.43 (41.88)	246.57 (108.07)	3.46 (1.00)	1.58% (0.69)	24.61 (0.38)	24.74 (0.60)	24.43 (0.26)	3	2	2

Table 2A-4. Summary information for Flathead Catfish gonadosomatic index values broken down by treatment, sex, and age group: minimum, maximum, mean, standard deviation (SD), standard error (SE), and 95% confidence intervals provided. All dissected Flathead Catfish were considered to be within the juvenile life stage.

Temperature,	Sex	Age, years	n	Min	Max	Mean	SD	SE	Lower CI	Upper CI
17	F	1	1	0.14	0.14	0.14	NA	NA	NA	NA
17	F	2	8	0.13	0.32	0.22	0.05	0.02	0.18	0.26
17	F	3	9	0.21	1.36	0.58	0.39	0.13	0.31	0.85
17	F	NA	1	0.20	0.20	0.20	NA	NA	NA	NA
17	M	1	1	0.03	0.03	0.03	NA	NA	NA	NA
17	M	2	7	NA	NA	NA	NA	NA	NA	NA
17	M	3	3	NA	NA	NA	NA	NA	NA	NA
17	U	1	1	NA	NA	NA	NA	NA	NA	NA
17	U	2	1	NA	NA	NA	NA	NA	NA	NA
17	U	3	1	NA	NA	NA	NA	NA	NA	NA
22	F	1	3	0.11	0.23	0.16	0.06	0.04	0.08	0.23
22	F	2	6	0.17	0.91	0.36	0.28	0.11	0.12	0.59
22	F	3	3	0.27	0.51	0.35	0.13	0.08	0.20	0.51
22	F	NA	2	0.12	0.30	0.21	0.13	0.09	0.03	0.40
22	M	1	4	NA	NA	NA	NA	NA	NA	NA
22	M	2	8	NA	NA	NA	NA	NA	NA	NA
22	M	3	5	0.15	0.29	0.20	0.06	0.03	0.14	0.25
22	M	4	1	NA	NA	NA	NA	NA	NA	NA
22	U	1	2	NA	NA	NA	NA	NA	NA	NA
22	U	2	2	NA	NA	NA	NA	NA	NA	NA
22	U	3	2	NA	NA	NA	NA	NA	NA	NA
25	F	1	2	0.28	0.30	0.29	0.01	0.01	0.27	0.30
25	F	2	9	0.27	0.92	0.46	0.19	0.06	0.33	0.59
25	F	3	7	0.39	0.79	0.57	0.14	0.05	0.47	0.68
25	F	5	1	1.35	1.35	1.35	NA	NA	NA	NA
25	M	1	2	NA	NA	NA	NA	NA	NA	NA
25	M	2	17	NA	NA	NA	NA	NA	NA	NA
25	M	3	5	0.12	0.29	0.18	0.07	0.03	0.12	0.24
25	M	4	2	0.22	0.25	0.23	0.02	0.01	0.21	0.26
25	U	1	2	NA	NA	NA	NA	NA	NA	NA
25	U	2	6	NA	NA	NA	NA	NA	NA	NA
25	U	3	2	NA	NA	NA	NA	NA	NA	NA

Table 2A-5. Post-hoc analysis using least square means for multiple comparison (Tukey-adjusted comparison) to examine differences in gondosomatic index values between five age groups of juvenile Flathead Catfish. Mean gonadosomatic index values were different between age groups based on $\alpha = 0.05$

Age	Estimate	SE	df	z-ratio	p-value
1-2	-0.13	0.07	Inf	-1.88	0.331
1-3	-0.25	0.07	Inf	-3.54	**0.004**
1-4	-0.16	0.15	Inf	-1.07	0.824
1-5	-1.05	0.20	Inf	-5.29	**< 0.001**
2-3	-0.12	0.05	Inf	-2.69	0.055
2-4	-0.03	0.14	Inf	-0.25	0.999
2-5	-0.93	0.19	Inf	-4.85	**< 0.001**
3-4	-0.09	0.14	Inf	0.61	0.973
3-5	-0.81	0.19	Inf	-4.30	**< 0.001**
4-5	-0.89	0.23	Inf	-3.84	**0.001**

Table 2A-6. Summary of mean and standard deviation (SD) of temperature (º C) and dissolved oxygen level (mg/L) for each tank throughout the duration of the experiment.

System	Tank	n	Mean ± (SD) Temperature, º C	Mean ± (SD) Dissolved Oxygen, mg/L
1	A	134	16.96 (0.53)	9.20 (0.43)
1	B	134	16.95 (0.52)	8.99 (0.40)
1	C	134	16.94 (0.51)	8.90 (0.45)
1	D	134	16.94 (0.52)	9.04 (0.41)
2	A	134	21.93 (0.63)	8.06 (0.48)
2	B	134	21.95 (0.56)	7.95 (0.44)
2	C	134	21.95 (0.55)	7.85 (0.41)
2	D	134	21.93 (0.54)	8.05 (0.40)
3	A	106	24.77 (0.43)	7.57 (0.47)
3	B	106	24.77 (0.42)	7.44 (0.44)
3	C	106	24.76 (0.42)	7.94 (4.84)
3	D	106	24.71 (0.40)	7.59 (0.44)

Table 2A-7. Summary information (count of trials (N), mean, standard deviation (SD), standard error (SE), and 95% confidence intervals (CI)) on the proportions of the meal remaining in the stomach at each digestion time interval (2h, 4h, 6h, 8h, 12h, 16h, 24h, 36h, and 48h) among treatments (17º C, 22º C, and 25º C).

Treatment Temperature, ºC	Digestion Time Interval	N	Mean Proportion of Meal Remaining	SD	SE	Lower CI	Upper CI
17	2	3	0.9092	0.1128	0.0651	0.7761	1.0423
17	4	3	0.8027	0.0564	0.0326	0.7360	0.8694
17	6	4	0.5942	0.0833	0.0416	0.5091	0.6793
17	8	4	0.4697	0.0284	0.0142	0.4407	0.4987
17	12	4	0.3702	0.2311	0.1156	0.1338	0.6066
17	16	3	0.3700	0.2765	0.1596	0.0436	0.6964
17	24	4	0.0039	0.0078	0.0039	-0.0041	0.0119
17	36	4	0	0	0	0	0
17	48	4	0	0	0	0	0
22	2	4	0.8143	0.1263	0.0632	0.6850	0.9436
22	4	4	0.6173	0.1503	0.0752	0.4635	0.7711
22	6	5	0.6104	0.2399	0.1073	0.3909	0.8299
22	8	5	0.3880	0.2112	0.0945	0.1947	0.5813
22	12	4	0.1162	0.2148	0.1074	-0.1035	0.3359
22	16	4	0.1030	0.2060	0.1030	-0.1077	0.3137
22	24	4	0.0314	0.0627	0.0314	-0.0328	0.0956
22	36	4	0	0	0	0	0
22	48	4	0	0	0	0	0
25	2	6	0.8756	0.1198	0.0489	0.7756	0.9756
25	4	6	0.7203	0.1675	0.0684	0.5804	0.8602
25	6	6	0.3739	0.2219	0.0906	0.1886	0.5592
25	8	6	0.2315	0.1811	0.0739	0.0804	0.3826
25	12	6	0.0673	0.1525	0.0623	-0.0601	0.1947
25	16	6	0.0195	0.0478	0.0195	-0.0204	0.0594
25	24	6	0	0	0	0	0
25	36	6	0	0	0	0	0
25	48	7	0	0	0	0	0

Table 2A-8. Count of Flathead Catfish (n = 126) that consumed a Goldfish broken down by one-hour time intervals over a 24-hour period. Lighting is dummy coded ("0" = lights off and "1" = lights on).

Time of Day	Count	Light_dummy
0045-0144	7	0
0145-0244	6	0
0245-0344	14	0
0345-0444	6	0
0445-0544	11	0
0545-0644	1	1
0645-0744	0	1
0745-0844	1	1
0845-0944	1	1
0945-1044	4	1
1045-1144	2	1
1145-1244	2	1
1245-1344	2	1
1345-1444	5	1
1445-1544	5	1
1545-1644	6	1
1645-1744	3	1
1745-1844	26	0
1845-1944	6	0
1945-2044	1	0
2045-2144	3	0
2145-2244	4	0
2245-2344	6	0
2345-0044	4	0

Appendix 3. Summary of wet weight to dry weight conversion and caloric content of Goldfish subsample.

Table A3-1. Goldfish subsample used to predict dry weight (n = 28) and initial calories (n = 17) of Goldfish fed to Flathead Catfish.

UID	Wet Weight	Dry Weight	Pellet 1 cals/g DW	Pellet 2 cals/g DW	Pellet 3 cals/g DW	Average cals/g DW
G1	2.25	0.41	3869.45	3705.81		3787.63
G2	5.20	1.03	3621.21	3841.32		3731.26
G3	4.95	1.00	3453.85	3626.03		3539.94
G4	3.10	0.58	3527.87	3670.61		3599.24
G5	3.29	0.60	3830.66	3661.22		3745.94
G6	4.65	0.90	3473.55	3615.48		3544.52
G7	1.54	0.24	3545.79	3577.68		3561.73
G8	1.86	0.31	3519.94	3426.9		3473.42
G9	6.67	1.26	3642.11	4465.67	4108.59	4072.12
G10	4.65	0.94	3707.49	3812.44		3759.97
G11	2.41	0.44	3565.67	3554.28		3559.98
G12	2.00	0.33	3801.3	3902.89		3852.1
G13	2.13	0.39				
G14	2.17	0.39	3645.19	3935.9		3790.55
G15	2.47	0.44	3925.38	3910.35		3917.87
G16	1.28	0.23				
G17	3.38	0.61				
G18	5.81	0.99	4169.63	3375.51		3772.57
G19	4.82	0.80	3846.45	3937.48		3891.97
G20	3.56	0.63				
G21	2.44	0.40				
G22	1.75	0.27				
G23	3.94	0.64	4210.71	4354.68		4282.69
G24	4.92	0.82				
G25	3.39	0.58				
G26	2.34	0.41				
G27	2.57	0.44				
G28	1.85	0.29				

Appendix 4. Length weight relationship of sampled juvenile Flathead Catfish and examination of relative weight (Wr) among treatments.

Figure A4-1: Weight-length relationship of our sample using the formula:

$$log_{10}(W_i) = log_{10}(\alpha) * \beta log_{10}(L_i) + \varepsilon_i,$$

where W_i is the individuals weight, L_i is the individuals length, β is an estimate of the slope, $\log_{10}(\alpha)$ is an estimate of the intercept, and ε_i is an additive error term. Using this equation, we predicted $\log_{10}(W)$ given $\log_{10}(L)$ which was then anti-logged to a more useful prediction of W_i. Followed the procedures outlined in Ogle (2016).

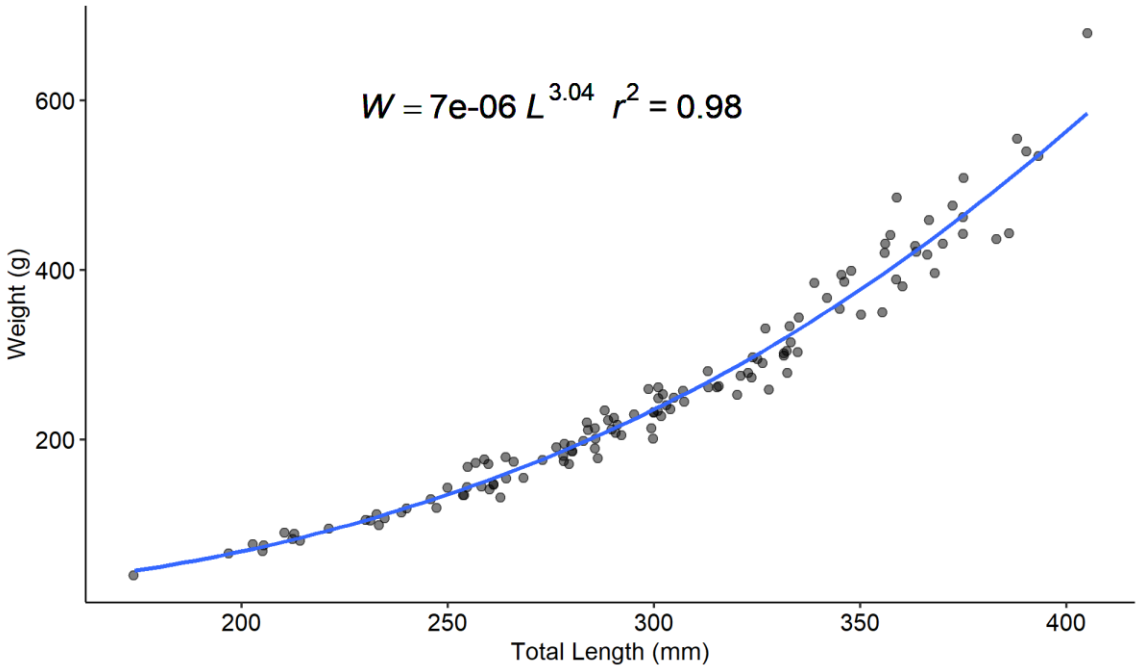
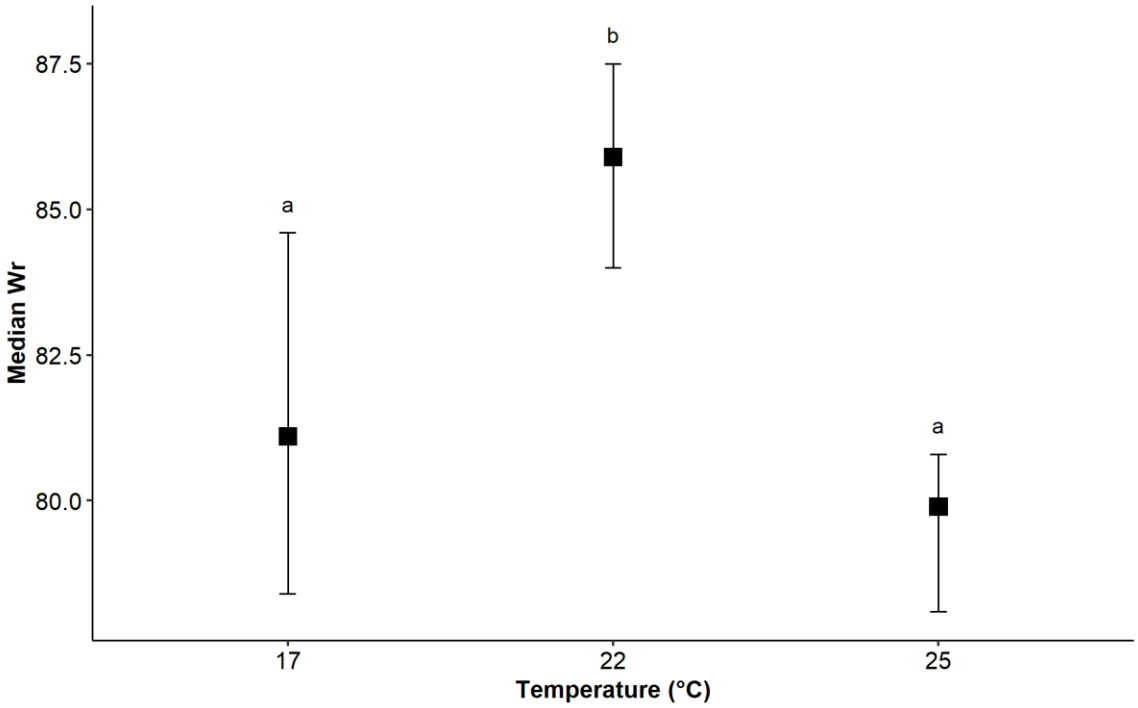


Figure A4-2: A Kruskal-Wallis Test was conducted to examine the differences on relative weights (Wr) for each individual at time of dissection according to treatment groups. Significant differences (Chi square = 20.02, $p < 0.001$, df = 2) were found among the three treatments (17º C, 22º C, and 25º C). Based on a Dunn Test, there was differences in condition between treatments of 17º C to 22º C ($z = -2.45$, $p < 0.001$) and 22º C to 25º C ($z = 4.47$, $p < 0.0001$), but not 17º C to 25º C ($z = 1.64$, $p < 0.101$.



Appendix 5. Raw data of each dissected Flathead Catfish in Chapter 2 and Chapter 3.

UID	Temperature (° C)	FHCF Total Length, mm	FHCF Weight, g	FHCF Gonad Weight, g	Sex	GF Weight, g	Digestion Time	Stomach Content Weight, g (WW)	Intestinal Content Weight, g (WW)	Stomach Content Weights, g (DW)	Intestinal Content Weight, g (DW)	Gonad Content Weight, g (DW)	Stomach calories calories/g DW	Intestine calories calories/g DW	Gonad calories calories/g DW
015	17	342	367.3	NA	U	1.96	12	0.24	1.39	0.03	0.15	NA	3094.9	2129.47	NA
016	25	278	195.1	NA	U	2.19	4	1.76	0.62	0.32	0.06	NA	5005.83	2770.08	NA
019	25	390	540.2	NA	U	1.72	16	0.07	0.53	0	0.1	NA	0	1562.44	NA
020	25	357	441.2	5.96	F	2.05	12	0	0.61	0	0.12	0.99	0	1835.79	5632.3
023	22	210	90.6	NA	U	1.72	8	0.43	0.43	0.06	0.09	NA	3514.35	2283.56	NA
024	25	286	213.7	NA	U	1.69	8	0	0.85	0	0.1	NA	0	1913.75	NA
028	22	290	225.7	NA	U	1.65	12	0	0.42	0	0.09	NA	0	1823.64	NA
031	22	327	331.3	0.74	M	2.63	16	0	0.53	0	0.12	NA	0	2048.83	NA
033	17	301	233.8	1.1	F	1.62	2	1.62	0.24	0.21	0.01	0.15	4181.6	0	5107.68
035	25	356	420.1	NA	U	1.4	12	0	0.45	0	0.1	NA	0	1808.55	NA
036	22	375	508.6	NA	U	3.64	4	2.51	1.35	0.42	0.1	NA	4698.8	2532.06	NA
037	22	359	485.6	NA	U	3.67	36	0	0.23	0	0.02	NA	0	3214.37	NA
038	25	295	230.2	NA	U	3.32	24	0	0.69	0	0.17	NA	0	2171.34	NA
041	25	355	350.3	2.76	F	3.05	48	0	0.07	0	0	0.37	0	0	5111.49
042	25	335	344	2.05	F	2.4	2	2.4	0.22	0.36	0.02	0.29	4087.3	0	5052.3
045	25	261	147.4	NA	U	2.06	48	0	0	0	0	NA	0	0	NA
046	22	284	220.6	0.38	F	2.95	6	1.65	1.03	0.22	0.15	0.06	4706.93	2411.29	4899.52
049	25	290	212.1	0.76	F	3.12	2	2.5	0.06	0.47	0	0.11	4755.25	0	5185.81
050	17	364	421.5	NA	M	2.55	24	0.13	0.91	0	0.15	NA	0	2065.33	NA
051	17	313	261.7	NA	U	4.46	8	2.64	1.29	0.38	0.2	NA	4197.87	3028.77	NA
052	25	280	186.2	0.72	F	2.76	16	0	0.64	0	0.14	0.1	0	1702.94	5059.59
053	22	366	418.2	1.19	F	2.99	8	0.71	1.22	0.06	0.16	0.17	4372.35	1889.25	5022.02
054	25	276	191.2	NA	U	2.98	24	0	0.29	0	0.08	NA	0	1729.63	NA
056	25	321	275.7	NA	M	2.14	6	0.59	0.75	0.09	0.1	NA	3539.66	2282.25	NA
057	25	279	171.3	0.7	F	3.66	36	0	0.1	0	0.03	0.09	0	1517.4	4984.8
059	25	292	205.5	0.75	F	1.74	36	0	0.08	0	0.02	0.1	0	0	5099.3
060	17	356	430.8	NA	M	2.28	36	0	0.58	0	0.12	NA	0	1832.16	NA
061	22	305	249.5	0.82	F	2.33	12	0.05	0.96	0	0.13	0.11	0	2087.34	5051.62
062	22	346	386.5	NA	M	2.37	24	0	0.74	0	0.11	NA	0	2146.94	NA
064	22	359	388.8	1.97	F	1.69	24	0	0.75	0	0.11	0.27	0	2242.63	5110.8
065	25	405	679.2	3.82	F	3.07	16	0.1	0.78	0	0.16	0.51	0	1968.9	5029.41
067	25	383	436.8	1.94	F	3.04	24	0	0.71	0	0.14	0.25	0	1840.62	5181.05
068	25	286	178.4	0.48	F	4.98	48	0	0.11	0	0.01	0.07	0	0	5041.58
070	22	212	82.8	0.1	F	1.99	36	0.1	0.13	0	0	0.02	0	0	5225.55
071	22	240	119.1	NA	U	3.26	6	2.32	0.28	0.38	0.04	NA	4377.55	2359.01	NA
072	17	259	176.6	NA	M	2.12	16	0.59	0.55	0.09	0.09	NA	4197.28	2409.23	NA
077	25	325	294.8	NA	M	2.53	36	0	0.01	0	0	NA	0	0	NA
078	25	324	273.2	NA	M	3.63	4	2.13	0.37	0.29	0.06	NA	3871.62	2323.66	NA
079	22	289	222.9	NA	M	3.38	16	0.01	0.62	0	0.12	NA	0	1994.44	NA
084	22	221	95.5	0.29	F	2.94	4	2.02	0.35	0.4	0.04	NA	4085.67	2598.41	NA
085	25	261	146.8	NA	U	3.37	2	2.95	0.12	0.6	0.01	NA	4654.9	0	NA
086	25	258	145	NA	U	3.04	48	0	0.01	0	0	NA	0	0	NA
087	17	328	259.4	0.53	F	4.97	36	0.03	1.08	0	0.29	0.07	0	1624.93	5037.08
090	22	345	394.6	0.6	M	2.47	48	0	0.05	0	0	0.08	0	0	5465.79
093	25	286	201.4	NA	M	4.26	12	0.13	1.13	0.02	0.2	NA	1558.75	1656.5	NA
094	22	280	193.3	0.5	F	3.43	12	1.84	0.81	0.27	0.11	0.07	5162.31	2480.83	4985.59
095	25	367	459	0.56	M	2.63	12	0	1.34	0	0.14	0.08	0	2187.14	5585.75
096	17	345	354.5	2.72	F	3.7	12	2.15	1.23	0.38	0.14	0.36	4836.26	2826.65	5111
097	22	213	88.8	0.1	F	3.63	16	1.52	0.74	0.27	0.11	0.01	4578.85	2772.57	5651.96
098	22	284	211.4	0.54	M	3.67	36	0	0.5	0	0.15	0.08	0	1544.61	5347.64
099	25	283	198.6	0.28	M	2.03	8	0.33	0.45	0.04	0.07	0.04	4621.45	2172.9	4870.71
101	22	313	280.7	0.46	M	2.81	8	1.89	0.93	0.28	0.12	0.06	4033.76	2188.63	5186.14
102	17	386	443.1	1.12	F	3.14	48	0	0.42	0	0.09	0.15	0	2609.93	4795.02
103	17	299	259.9	NA	M	3.17	16	0.73	1.43	0.1	0.21	NA	4707.36	2795.32	NA
106	25	247	120	NA	M	3.61	36	0	0.44	0	0.13	NA	0	1491.5	NA
107	25	260	141.7	NA	M	2.34	24	0	0.23	0	0.06	NA	0	1525.11	NA
113	25	254	134.7	0.77	F	4.35	4	3.23	0.47	0.74	0.05	0.1	5312.62	2769.09	5021.6

UID	Temperature (° C)	FHCF Total Length, mm	FHCF Weight, g	FHCF Gonad Weight, g	Sex	GF Weight, g	Digestion Time	Stomach Content Weight, g (WW)	Intestinal Content Weight, g (WW)	Stomach Content Weights, g (DW)	Intestinal Content Weight, g (DW)	Gonad Content Weight, g (DW)	Stomach calories calories/g DW	Intestine calories calories/g DW	Gonad calories calories/g DW
114	25	300	232.5	NA	M	2.59	16	0	0.88	0	0.14	NA	0	1828.37	NA
115	22	302	227.9	0.61	F	4.67	2	4.11	0.74	0.69	0.04	0.09	5235.48	3301.68	4996.41
116	22	302	253.9	0.53	M	2.39	4	1.39	0.61	0.17	0.06	0.07	3486.28	2740.72	5422.74
118	17	255	168.3	NA	M	4.49	2	3.74	0.92	0.82	0.04	NA	5403.83	4400.58	NA
119	25	197	66	NA	U	3.25	12	1.22	0.53	0.22	0.09	NA	5492.27	2326.34	NA
120	25	350	347.4	0.86	M	2.88	6	1.44	0.78	0.25	0.11	0.09	5253.71	2677.23	5434.15
121	22	388	555.2	0.88	M	3.68	2	3.34	1.28	0.66	0.06	0.11	5222.5	3548.24	5254.71
123	22	214	81.3	NA	M	4.56	6	3.28	0.62	0.8	0.06	NA	5738.61	3307.48	NA
124	25	268	155.1	NA	M	3.71	8	1.59	0.67	0.31	0.12	NA	5162.56	2683.51	NA
126	22	250	143.7	0.19	M	3.83	8	2.35	1.32	0.38	0.17	0.03	4039.55	2390.84	5460.91
127	25	278	175	1.25	F	1.81	16	0	0.61	0	0.16	0.17	0	1302.6	5206.43
128	25	307	258	0.57	M	2.99	36	0.01	0.21	0	0.01	0.08	0	0	5627.26
129	25	370	431.4	0.78	M	1.58	6	0	1.05	0	0.13	0.1	0	1831.57	5448.33
130	25	231	104.7	0.31	F	1.79	8	0.68	0.35	0.13	0.06	0.05	3055.86	2315.72	4809.78
133	17	333	314.8	4.28	F	3.06	4	2.69	1.08	0.46	0.08	0.84	3872.91	2402.22	5678.31
134	25	233	99.3	0.28	F	2.7	6	1.51	0.48	0.29	0.07	0.04	3883.02	2151.33	5337.16
135	25	324	297.4	0.69	M	1.94	8	0.56	0.86	0.06	0.12	0.1	3921.9	1733.31	5615.2
136	22	360	380.6	1.12	M	2.44	48	0.02	0.03	0	0.01	0.13	0	0	5310.06
140	25	301	249.1	0.57	M	3.37	2	2.25	0.37	0.4	0.04	0.08	3611.04	2809.96	5562.46
141	25	363	428.6	1.2	M	4.43	48	0.01	0.82	0	0.16	0.14	0	1368.86	5168.23
143	22	239	114.4	0.07	M	1.73	4	1.24	0.59	0.19	0.06	0.01	3747.07	2387.58	5323.29
146	22	301	261.9	0.41	M	2.16	6	1.56	0.63	0.25	0.06	0.06	4043.83	2293.21	5354.86
147	25	254	134.4	NA	M	2.82	2	2.52	0.2	0.48	0.02	NA	3246.06	0	NA
148	22	233	112.1	NA	M	5.61	24	1.05	0.72	0.13	0.17	NA	3585.93	1753.54	NA
149	17	320	252.9	0.59	F	3.9	8	2.52	0.96	0.36	0.11	0.08	3833.92	1980.08	4797.86
150	25	303	241	0.95	F	2.33	6	1.49	1.05	0.21	0.12	0.13	4036.84	1945.06	5203.86
151	25	307	244.7	0.54	M	3.51	4	2.62	0.89	0.44	0.1	0.08	4173.15	2604.9	5420.4
152	22	375	462.4	0.95	M	2.15	12	0.07	1.03	0.01	0.16	0.12	0	1727.51	5131.63
155	22	260	171.7	1.57	F	2.76	36	0	0.17	0	0.04	0.25	0	1483.48	5184.33
156	25	315	261.9	0.94	F	5.11	16	0.82	1.31	0.11	0.31	0.13	3156.4	1750.26	5125.41
157	25	393	534.1	2.82	F	5.28	4	4.15	0.87	0.66	0.12	0.37	4127.51	2610.15	5285.56
159	22	348	399.4	0.71	F	3.25	2	2.79	0.74	0.44	0.04	0.11	4029.57	3208.19	5029.21
161	25	304	235.8	0.51	M	2.87	12	0	1.01	0	0.17	0.05	0	1743.31	5260.52
162	25	333	333.6	0.66	M	4.86	24	0.14	0.73	0	0.16	0.08	0	1511.35	5253.08
163	25	375	442.8	0.58	M	6.59	4	4.77	0.82	0.89	0.12	0.09	3820.99	2186.3	5551.33
164	25	331	301.9	1.44	F	4.43	8	1.14	1.37	0.16	0.24	0.19	3681.72	2058.4	5062.51
165	22	205	68.5	NA	U	1.41	2	1.18	0.23	0.16	0.02	NA	3798.95	3487.16	NA
166	22	203	77.2	0.1	F	2.68	8	1.32	0.26	0.24	0.06	0.02	3442.43	2179.04	5211.7
167	17	332	279	0.62	F	2.18	6	1.8	1.24	0.25	0.08	0.09	3765.51	2304.91	4890.89
168	22	205	75.3	0.17	F	1.4	16	0	0.44	0	0.09	0.03	0	1605.05	5195.9
169	25	263	132	0.26	M	2.27	36	0.02	0.13	0	0.03	0.04	0	2086.25	5478.22
170	25	291	208.2	0.24	M	3.08	6	1.32	1.09	0.21	0.16	0.04	3195.44	2358.92	5793.2
173	22	335	303	0.54	M	5.31	48	0	0.94	0	0.19	0.06	0	1468.06	5421.45
176	25	332	304.9	0.87	M	4.22	2	3.99	0.75	0.72	0.07	0.09	4493.09	3265.03	5369.58
177	25	286	189.6	1.75	F	2.93	24	0	0.57	0	0.15	0.24	0	1283.98	4940.97
178	22	273	176.2	0.49	F	4.33	24	0	1.15	0	0.26	0.07	0	1498.51	5005.42
179	17	339	384.8	0.81	F	3.35	24	0.08	1.17	0	0.23	0.11	0	2028.97	4886.82
180	17	331	299.1	0.45	M	1.58	48	0.19	0.33	0	0.07	0.07	0	2043.05	5443.32
181	22	235	107.4	0.06	M	1.51	48	0	0	0	0	0.01	0	0	NA
182	25	316	263	0.75	M	2.88	48	0	0.50	0	0.12	0.1	0	1334.55	5310.8
183	25	299	213.3	0.54	M	3.80	48	0.05	0.61	0	0.12	0.07	0	1251.79	5481.9
187	17	278	181.2	0.37	F	2.11	8	1.02	0.85	0.16	0.08	0.06	3860.72	2498.45	4960.15
190	17	174	40.5	0.13	F	2.42	16	1.78	0.35	0.29	0.09	0.02	4361.89	2691.25	4929.08
192	17	368	396.5	0.96	F	4.03	48	0	1.16	0	0.26	0.12	0	1613.49	5127.29
194	17	230	105.8	0.23	F	2.31	48	0	0.61	0	0.17	0.04	0	1493.35	5026.3
195	17	326	290.1	1.25	F	3.33	6	2.93	1.70	0.38	0.11	0.16	3937.72	1961.28	5071.99
196	22	264	154.8	0.17	M	1.26	6	0.55	0.56	0.07	0.07	0.02	3215.94	2220.98	5580.33
197	17	300	201.4	0.11	M	2.75	24	0.07	0.98	0	0.21	0.02	0	2247.35	4698.07
202	17	372	475.8	1.89	F	3.57	24	0.04	1.40	0.01	0.26	0.26	0	2129.76	5050.96
204	17	280	186.7	0.56	F	4.63	36	0	1.17	0	0.24	0.07	0	1816.25	5183.61
205	17	300	232.2	0.23	M	2.82	6	1.94	1.48	0.30	0.10	0.03	3622.9	2295.8	5454.09

UID	Temperature (° C)	FHCF Total Length, mm	FHCF Weight, g	FHCF Gonad Weight, g	Sex	GF Weight, g	Digestion Time	Stomach Content Weight, g (WW)	Intestinal Content Weight, g (WW)	Stomach Content Weights, g (DW)	Intestinal Content Weight, g (DW)	Gonad Content Weight, g (DW)	Stomach calories calories/g DW	Intestine calories calories/g DW	Gonad calories calories/g DW
206	17	266	174.4	0.05	M	2.96	12	2.36	1.09	0.29	0.11	0.01	4142.9	2012.01	NA
207	17	291	217.5	0.44	M	4.29	2	4.02	0.66	0.74	0.04	0.06	4555.21	4636.36	5433.01
208	17	264	179.6	NA	M	2.90	4	2.3	0.76	0.38	0.04	NA	3770.45	2703.51	NA
209	17	323	278.5	2.88	F	5.63	4	4.17	0.28	0.86	0.02	0.55	4643.98	2030.07	5486.27
213	17	288	234.9	0.41	F	2.52	12	0.94	1	0.12	0.14	0.06	4370.21	2239.64	5008.02
214	17	246	130.1	0.18	F	2.76	36	0.02	0.65	0	0.19	0.02	0	2649.78	5222.08
215	17	255	144	NA	U	1.10	6	0.76	0.62	0.08	0.05	NA	4151.52	2787.52	NA
216	17	257	172.5	0.23	F	0.90	8	0.59	0.59	0.06	0.04	0.04	3524.13	2587.67	5254.64